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An International Journal of Genetics

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An International Journal of Genetics

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HEREDITY VOLUME 15 PART 1 AUGUST 1960

INDUCED MUTATIONS AT THE V^{by} LOCUS OF *TRIFOLIUM REPENS*

I. EFFECTS OF ACUTE, CHRONIC AND FRACTIONATED DOSES OF GAMMA RADIATION ON INDUCTION OF SOMATIC MUTATIONS

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1. INTRODUCTION

CONSIDERABLE attention has been devoted recently to the possibility of increasing the frequency of occurrence of somatic mutations in plants by exposing them to ionising radiations, and to this end a tremendous number of samples of different species and varieties have been treated. Very little basic information, however, is available regarding the induction of these mutations, and in this paper results are presented of the comparative effects of acute, fractionated and chronic doses of gamma radiation on somatic mutation rates. Such comparative studies have previously been made in plants only in terms of induced chromosome breakage (*e.g.* Lane, 1952 ; Steffenson and Arnason, 1954 ; Sax *et al.*, 1955) and of growth, survival and mutation induction after exposure of seeds (Kaplan, 1951 ; Schooler *et al.*, 1957). Cuany *et al.* (1958) have exposed clonal lines of *Antirrhinum majus* heterozygous for three colour genes, to one dose of acute X-irradiation and to chronic gamma irradiation at various dose rates, but no data are available which allow a comparison of the effects of the two treatments.

The studies reported here were concerned with the somatic mutations induced by gamma radiation at the leaf marking locus of diploid white clover (*Trifolium repens*). The allele studied was V^{by}, one of eleven at this locus (Brewbaker and Carnahan, 1956), which results in the production of a phenotype distinguished by the presence of a broken white V surmounted by a yellow tip (plate I, fig. 1). No crossing-over has been reported between the constituent parts of the locus. Two clones derived from two sister plants and heterozygous for this allele and the recessive allele *v*, which in the homozygous condition results in the complete absence of a leaf mark, were produced for the series of experiments. Most allelic compounds at this locus, apart from those involving *v*, exhibit mosaic dominance, though one allele—V^{B}—is partially or completely dominant to four other alleles (Brewbaker, 1955).

Though the primary aim of the study was an elucidation of the effect of dose fractionation, it was also of interest to note the range of mutant phenotypes produced in this somatic tissue. Since the gene being investigated is already known to exist in allelic forms of varying

expression, there seemed a good opportunity of producing and detecting new phenotypic expressions, and as the locus probably has little physiological effect, mutants would not be at a disadvantage. The locus is also advantageous for mutation experiments, in that large numbers of leaves can easily be grown and examined for mutations, but it has a disadvantage in that mutation rates can not be determined, as the number of potential units of mutation is unknown. The only value that can be determined is the frequency of occurrence of leaves showing mutations.

The term mutation of the leaf marking allele is used in its broadest sense. True gene or point mutations cannot here be distinguished from partial or whole chromosome losses, and it is quite likely that chromosomal deficiencies represent a large part of the induced mutations. The time of appearance after treatment of the first mutations varied according to the conditions of growth, from four weeks in summer to ten weeks in winter. Initially mutations were recognised as an absence of, or a change in, a small portion of the leaf mark. Later leaflets had larger segments of mutant tissue, then whole leaflets and ultimately whole leaves and tillers were mutant. The standard scoring procedure was to count mutant leaf marks irrespective of their size, as one mutational event, and if two or more associated leaflets or leaves had similar mutant phenotypes then this was scored as one mutation. The total numbers of leaves produced were recorded and all except those exhibiting mutations removed from the plant after each time of scoring. Mutant leaves were left and marked in order that associated mutations arising later from the same mutational event could be recognised.

No spontaneous mutation was found in the control plants utilised in all the experiments considered here, (*ca.* 10,000 leaves) and none has been found in plants used for other experiments.

2. IRRADIATION TECHNIQUE

All irradiations were undertaken in a cell specially designed for plant irradiation, at the Wantage Radiation Laboratories (Piggot, in press). This cell which is temperature controlled above ambient to $\pm 1^{\circ}$ C., is large enough to allow a working space around the source. The 120 curie Co^{60} source is stored in a lead " coffin " (plate II) sited on the floor of the cell, and can be raised vertically by remote control through a guide tube to any height up to 100 cm. above the turntables on which the plants are located. The turntables are situated on six radial arms in a horizontal plane on the top surface of the lead " coffin." Dose rates of 600 to 6000 rads per hour are provided accurately, reproducibly and uniformly—the rate being controlled by the distance of the turntables from the central guide tube; uniformity throughout the volume being irradiated is ensured by rotating the turntables at 1 r.p.m.

3. THE DOSE-RESPONSE CURVE

Six uniform tillers of the heterozygous material, each 4-6 in. long and having initially a similar number of leaves, constituted one treatment sample. These were defoliated and then exposed at room temperature, at a dose rate of 1000 rads per hour, to one of the following doses of gamma rays :—

500, 1000, 1500, 2000, 2500, 3100 and 4000 rads.

The plants were irradiated in February, and were first examined 28 days later, and again on the 42nd, 56th and 70th days after irradiation. The total numbers of mutant and normal leaves from all the scoring dates are given in table I. Up to 1500 rads there was very

TABLE 1

Dose-response experiment : numbers of normal and mutant leaves in irradiated Trifolium repens

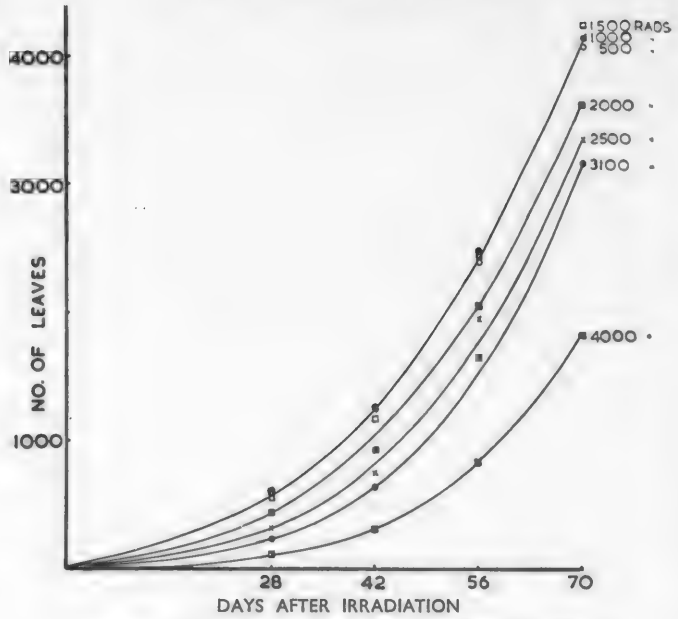
Dose in rads	Total no. leaves	Leaves with mutations		No. mutants repeated
		Number *	Percentage	
500	4097	9	0·22	0
1000	4176	28	0·67	1
1500	4234	59	1·39	10
2000	3657	79	2·16	15
2500	3358	89	2·65	13
3100	3213	92	2·86	22
4000	1891	75	3·97	15

* Repeats scored as 1 mutational event (=occurrence of same mutation on successive leaves of one tiller).

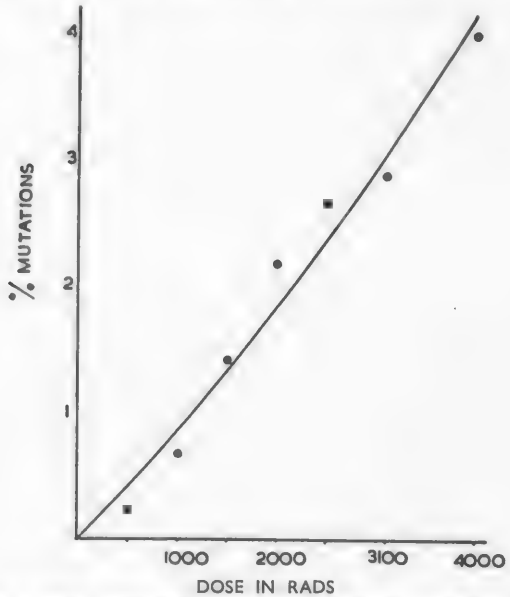
little effect on leaf production, but above this dose total leaf numbers fell markedly (text-fig. 1).

A considerable number of mutations of the leaf mark were observed after exposure to γ radiation. At the lowest dose used, 500 rads, there were 0·22 per cent. and at the highest, 4000 rads, there were 3·97 per cent. of leaves with mutations (text-fig. 2). A considerable number of the mutations observed were small gaps either in the white V or yellow tip. As some of these gaps could conceivably be missed due to their small size, it was decided to include in the tables, graphs and statistical analyses only those mutational events which affected a half-leaflet or larger portion of the tissue. Later analyses showed that the treatment responses in terms of half-leaflet mutations and the smaller gaps were the same, and so the latter were included in the last of the three experiments discussed here.

Statistical analyses of the data were undertaken, to elucidate the nature of the dose response. This involved fitting linear and quadratic functions to the percentage mutant leaves and the dose. For a straight line, the formula is $y=bx+c$ where b and c are estimated by minimising the sums of squares of the errors, and x is the dose in rads. As the



TEXT-FIG. 1.—Effect of γ radiation on leaf production (*T. repens*).



TEXT-FIG. 2.—Effect of γ radiation on induction of mutations (V locus in *T. repens*).

Plate I

FIG. 1.—Leaves of *T. repens* homozygous for the recessive allele "*v*" (left) and heterozygous for "V^{bv}" and "*v*" (right).

FIG. 2.—Mutant leaves exhibiting from left to right, the two "yellow tip" and the "broken V" phenotypes respectively.

FIG. 3.—Mutant leaves with white V reduced to spots and the yellow tip smaller in size.

FIG. 4.—Mutant leaves with various numbers of leaflets exhibiting the "faint mark" phenotype.

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control mutation rate is zero, $c=0$. This gives the relation between the observations and the dose in rads of $y=0.0000097962\,x$.

For the quadratic function the formula is $y=ax^2+bx+c$, again a and b to be estimated, x the dose in rads, and c being omitted as the control value is zero. This gives a relationship of, $y=0{\cdot}0000092039\,x+0{\cdot}000000001912\,x^2$.

Substituting for x in these equations, the numbers of mutant leaves expected at each dose were determined, and agreement between observed and expected values tested by calculating χ^2.

Testing the goodness of fit with a straight line, $\chi^2_{(6)}=12{\cdot}002$ and $P=0{\cdot}10$ to $0{\cdot}05$.

TABLE 2

Dose-response experiment. Frequencies of mutant phenotypes

Dose in rads	Loss of complete mark (no. and per cent.)	Loss of white V (no. and per cent.)	Loss of yellow tip (no. and per cent.)	White V reduced to spots (no. and per cent.)	White V reduced in intensity (no. and per cent.)
500	1	2	0	0	0
	0·024	0·049	0	0	0
1000	1	2	0	0	0
	0·024	0·048	0	0	0
1500	7	3	1	2	0
	0·165	0·071	0·024	0·048	0
2000	9	0	2	3	8
	0·246	0	0·055	0·082	0·220
2500	9	8	0	3	4
	0·268	0·238	0	0·089	0·119
3100	10	10	1	3	8
	0·311	0·311	0·031	0·093	0·249
4000	8	7	0	9	8
	0·423	0·370	0	0·476	0·423

Testing the goodness of fit with a quadratic curve, $\chi^2_{(5)}=10{\cdot}751$ and $P=0{\cdot}10$ to $0{\cdot}05$. This shows that the dose-response relationship gives a similar fit to quadratic and linear functions.

A linear relationship has been found for most classes of point mutations studied, over a great range of doses and a great variety of organisms (Muller, 1954). Such a relationship indicates that each mutation is a one-hit event. The results of this and the fractionation experiments discussed indicate that at least some of the mutational events recorded here were multi-hit events. At the highest doses studied it is possible that there was a non-recovery of certain mutations due to accompanying lethals—this would result in a flattening of the quadratic curve and suggest a linear relationship, in other words, the quadratic component might well be greater for the induction of mutations than for the visible mutants scored. The different types of mutations observed are shown in plate I, figs. 2-4, and their relative frequencies in table 2, the data being based only on those instances where whole leaflets were mutant and there could be no doubt as to

their exact nature and extent. Since the rate of occurrence of such whole leaflet mutant types was very low statistical analyses of the dose response in terms of the various mutant phenotypes was not justified.

4. EFFECT OF DOSE-FRACTIONATION

In this experiment it was required to compare firstly the effect of splitting a given dose into two or more fractions, and secondly the effect of varying the time intervals between fractions.

Nine similar-sized tillers of each of the two heterozygous clones constituted one treatment sample in this experiment. The tillers were defoliated and then at room temperature, exposed to one of the treatments listed in table 3, at a dose rate of 1000 rads per hour. The

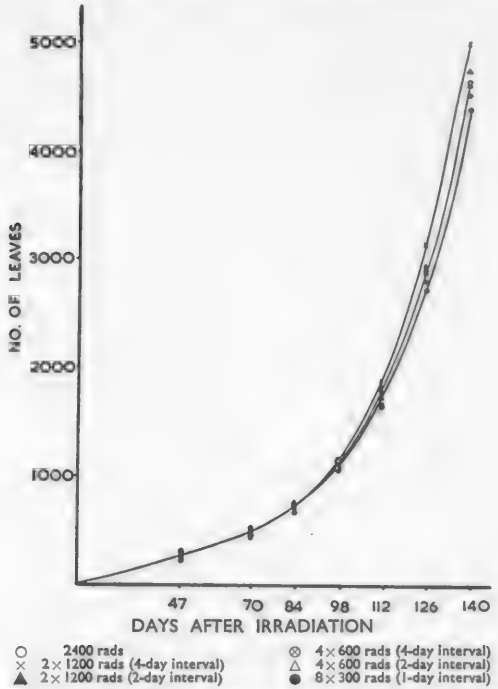
TABLE 3

Numbers of normal and mutant leaves after acute and fractionated doses.

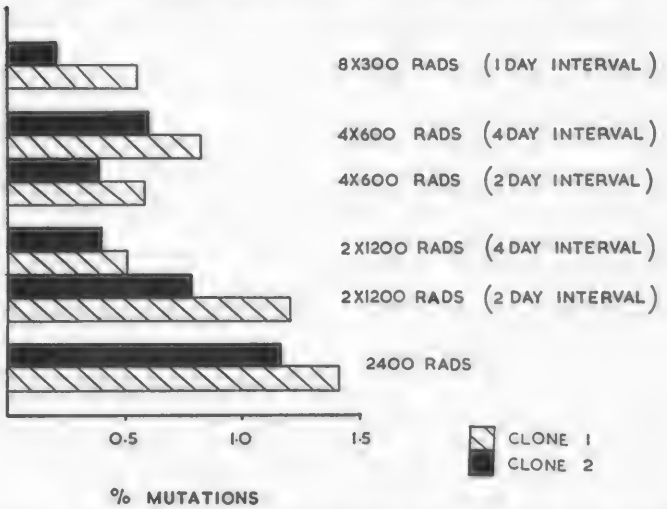
Dose in rads	Time interval between fractions (days)	Clone	Total no. leaves	Leaves with mutations		No. mutants repeated
				Number *	Percentage	
2400	—	I	2121	30	1·41	5
		II	2575	30	1·17	1
		I+II	4696	60	1·28	6
2×1200	2	I	2355	28	1·19	2
		II	2320	18	0·78	2
		I+II	4675	46	0·98	4
2×1200	4	I	2360	12	0·51	1
		II	2772	11	0·40	2
		I+II	5132	23	0·45	3
4×600	2	I	2252	13	0·58	0
		II	2540	10	0·39	1
		I+II	4792	23	0·48	1
4×600	4	I	2067	17	0·82	1
		II	2339	14	0·60	1
		I+II	4406	31	0·70	2
8×300	1	I	2189	12	0·55	3
		II	2388	5	0·21	0
		I+II	4577	17	0·37	3

* Repeats (occurrence of same mutation on successive leaves of tiller) are scored as one mutational event.

treatments were carried out in November ; in the intervals between fractions and at all times after irradiation plants were kept in a heated greenhouse. Scoring of the material for leaf numbers and mutations commenced after ten weeks, and after that time at fortnightly intervals for another ten weeks. The total number of leaves and mutations over all scoring dates are given in table 3, and text-figs. 3 and 4. There was no differential effect of fractionation on leaf production—but a very considerable effect on induction of mutations. The two clones will



TEXT-FIG. 3.—Effect of dose fractionation on leaf production (*T. repens*).



TEXT-FIG. 4.—Effect of dose fractionation and differing time intervals between fractions on induction of somatic mutations by γ radiation (V locus in *T. repens*; irradiated in November).

be considered together initially and mean values for both genotypes utilised in the comparisons of the effects of fractionation. 2400 rads given in one acute treatment produced a mean frequency of 1·28 per cent. mutations; if the total dose was given in two fractions of 1200 rads separated by a two-day interval, there was a slight, but not significant, decrease to 0·98 per cent. (table 4). Separating the fractions

TABLE 4

χ^2 and probability values for differences in mutation frequencies

Comparison (χ^2 test)	Clone	χ^2	Probability
(*a*) Clone I *v.* Clone II		6·230	0·02—0·01
(*b*) All treatments	I	19·232	0·01—0·001
	II	25·249	<0·001
	I+II	44·481	<0·001
(*c*) 2400 *v.* 2×1200 (2-day interval)	I	0·436	0·7—0·5
	II	1·897	0·2—0·1
	I+II	2·333	0·5—0·3
(*d*) 2400 *v.* 4×600 (4-day interval)	I	3·314	0·1—0·05
	II	4·417	0·05—0·02
	I+II	7·731	0·05—0·02
(*e*) 2×1200 (2 day) *v.* 2×1200 (4 day)	I	6·523	0·02—0·01
	II	3·210	0·10—0·05
	I+II	9·733	0·01—0·001
(*f*) 4×600 (2 day) *v.* 4×600 (4 day)	I	0·949	0·5—0·3
	II	1·065	0·5—0·3
	I+II	2·014	0·5—0·3
(*g*) 2×1200 (2 day) *v.* 4×600 (2 day)	I	4·831	0·05—0·02
	II	3·036	0·10—0·05
	I+II	7·867	0·02—0·01
(*h*) 2×1200 (4 day) *v.* 4×600 (4 day)	I	1·692	0·2—0·1
	II	1·045	0·5—0·3
	I+II	2·737	0·3—0·2
(*i*) 4×600 (4 day) *v.* 8×300 (1 day)	I	1·170	0·3—0·2
	II	4·498	0·05—0·02
	I+II	5·668	0·10—0·05
(*j*) 2×1200 (2 day) *v.* 4×600 (4 day)	I	1·468	0·3—0·2
	II	0·496	0·5—0·3
	I+II	1·964	0·5—0·3

by an interval of four days resulted in a very significant decrease to 0·45 per cent. mutations. If some of the mutations were two-hit events, then it may be assumed that over two days, damaged sites produced by the first could interact with those produced by the second fraction, but if there was a four-day interval such an interaction could not occur. The small quadratic component in the dose-response function indicates that this non-interaction could not be the sole reason for the difference between the two- and four-day intervals, and no adequate explanation is available.

The 4×600 rads treatments, separated either by two- or four-day intervals gave different results. Both long and short intervals resulted in a similar yield of mutations. This indicates an effect of dose on a repair mechanism. After 1200 rads repair was delayed and interaction could occur over two days, whereas after 600 rads repair could occur in less than two days. An analogy can be made with the results of Wolff and Atwood (1954) who found that the delay in rejoining of chromosome breaks was dose dependent.

The 8×300 rads treatment, separated by one-day intervals, gave the lowest mean frequency of mutations of all, 0·37 per cent. The consistent reduction in the rate after fractionation could be attributed to several factors, including :—

(1) The two-hit nature of some of the events, and non-interaction between successive fractions ; but as explained previously, this factor is not likely to be of great importance.

(2) A retarding of cell division after the first fraction so that later fractions act on a population comprising more of resting, and hence of more resistant, cells. Again it is doubtful whether the variation in the sensitivity of cells at different stages of development can account entirely for the reduction observed on fractionation.

(3) The physiological effect of the first fraction of radiation may affect the sensitivity of cells to a later fraction (Lane, 1952).

The amount of physiological damage and induced lethality, as measured in terms of leaf production, was not influenced differentially by any of the fractionation treatments. It may be assumed from the previous experiment that a dose of 2400 rads results in a reduction of leaf production. Since the effect on leaf growth is probably multi-hit a reduction of damage might have been expected after fractionation, but the absence of any such reduction indicates that in direct contrast to the response of mutational events, a complete interaction of the effects of separate fractions was possible.

5. COMPARISON OF ACUTE, FRACTIONATED AND CHRONIC DOSES

In order to elucidate further the nature of the dose-fractionation phenomenon, and to obtain quantitative information on the comparative efficiencies of the different treatments, a further experiment was undertaken. Details of the treatments are given in table 5. Each treatment sample consisted of four similar tillers of clone I (see previous experiment). These were defoliated prior to treatment and exposed at room temperature at a dose rate of 1122 rads per hour, apart from the chronic irradiation, for which treatment the rate was 25 rads per hour. The plants were irradiated in May and maintained in a greenhouse between and after all treatments.

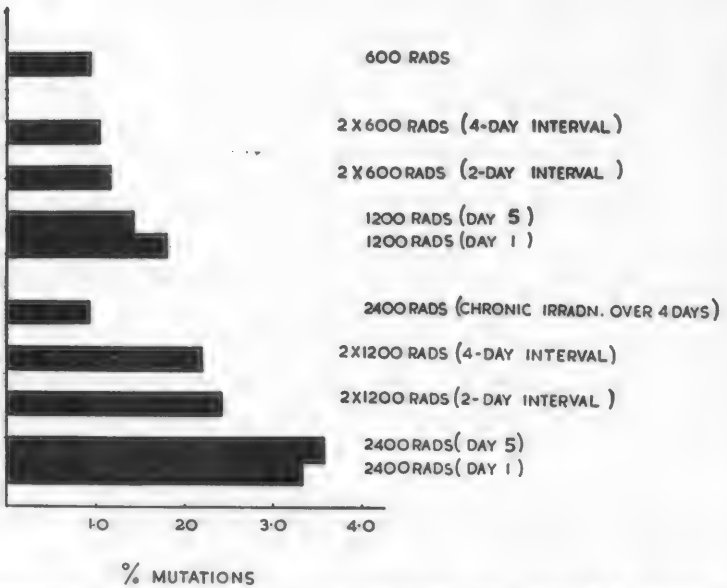
Numbers of leaves and mutations were first scored after five weeks and afterwards at three-weekly intervals for a further six weeks—total values for all scoring dates are given in table 5 and text-fig. 5.

The acute treatments, 2400 rads and 1200 rads were replicated—one on the first day and the other on the fifth day of the experiment, to check on any possible differences due to time of treatment. No

TABLE 5

Numbers of normal and mutant leaves after acute, fractionated and chronic doses

Dose in rads	Day irradiated	Total no. leaves	Leaves with mutations	
			Number	Percentage
2400	1	3671	122	3·32
2400	5	3254	116	3·57
2×1200	1 and 5	3247	71	2·19
2×1200	3 and 5	3187	77	2·42
2400 chronic	1 to 5	3575	33	0·92
1200	1	3129	56	1·79
1200	5	3581	51	1·42
2×600	1 and 5	3159	33	1·05
2×600	3 and 5	3602	42	1·17
600	1	3402	32	0·94



TEXT-FIG. 5.—Effects of acute, fractionated and chronic doses of γ radiation on the induction of somatic mutations (V locus in *T. repens*; irradiated in May).

significant differences were found due to time of treatment (table 6). Splitting the dose into two fractions of 1200 rads reduced the effect very considerably, but the time intervals of two or four days between fractions had no differential effect. This is in marked contrast to the

previous experiments and a possible explanation may lie in the different seasons at which the materials were treated and grown. Plants in this experiment were irradiated in May, those in the previous one in November. At the former time conditions of growth were at an optimum—damage induced by the first fraction could be repaired more quickly than in November and hence there would be less interaction between the two fractions. The two ×600 rads fractionation treatments were also similar, whether separated by two or four days. There was a 33 per cent. reduction in the number of mutations recovered when the 2400 rads dose was fractioned into two doses of 1200 rads and a 31 per cent. reduction from 1200 rads to two doses of 600 rads. The values obtained for two fractions of 1200 rads do not equal or

TABLE 6

χ^2 and probability values for differences in mutation frequencies

Comparison	χ^2	Probability
2400 (1st day) *v.* 2400 (5th day)	0·301	0·7—0·5
2400 (1st day) *v.* 2×1200 (2-day interval)	4·964	0·05—0·02
1200 (1st day) *v.* 1200 (5th day)	1·419	0·3—0·2
1200 (1st day) *v.* 2×600 (2-day interval)	4·537	0·05—0·02
1200 (5th day) *v.* 2×600 (4-day interval)	1·985	0·2—0·1
2×1200 (2-day interval) *v.* 2×1200 (4-day interval)	0·379	0·7—0·5
2×600 (2-day interval) *v.* 2×600 (4-day interval)	0·225	0·7—0·5
600 *v.* 2×600 (2-day interval)	0·945	0·5—0·3
1200 (1st day) *v.* 2×1200 (2-day interval)	3·013	0·1—0·05
1200 (5th day) *v.* 2×1200 (4-day interval)	5·645	0·02—0·01
2400 chronic *v.* 600	0·0065	0·95—0·90

even approach twice the value obtained with one treatment of 1200 rads. Similarly there was no significant difference between one dose of 600 rads and two doses of 600 rads separated by either two or four days. When 2400 rads were given as a chronic dose, at a rate of 25 rads per hour, the numbers of mutations recovered were not significantly different from that obtained with a single acute dose of 600 rads.

As in the previous experiment the explanation for the reduction in recoverable mutations after fractionation of the dose, or chronic exposure, may be found in a changed sensitivity of cells after the initial treatment. Irrespective of whether the events being scored are one-hit, or multi-hit, which can or cannot interact, the total number of mutations from two fractions should still equal twice the value obtained from one fraction alone, but this doubled value was not obtained.

6. EFFECT OF GENOTYPE ON MUTABILITY

Pronounced and consistent differences were noted in the radiosensitivity, as measured in terms of induced mutations at the V^{by} locus, of the two genotypes used in the first fractionation experiment (table 3 and fig. 4). The two clones used were propagated vegetatively from sister plants—the progeny of two common parents. Clone I

consistently produced more mutations than Clone II, $\chi^2_{(1)}=6{\cdot}23$, P $=0{\cdot}02$ to $0{\cdot}01$ (table 4). Such a pronounced difference in the sensitivity of one locus in plants of quite similar genetic background has not previously been reported.

7. MUTANT PHENOTYPES

The mutant phenotypes produced on irradiating tissue heterozygous for V^{by} and the recessive "no-mark" allele could be separated into two classes. The first included those in which either both or only one of the component parts of the mark had been lost (plate I, fig. 2) and the second, those exhibiting a modified form of mark (plate I, figs. 3 and 4). All mutations appeared completely viable, and whole tillers of mutant phenotypes were propagated.

In the first class, by far the most frequent, the loss of the whole mark simulated the double recessive *v v* phenotype and the loss of the yellow tip the V^B phenotype (Brewbaker, 1955). Artificial and natural V^B phenotypes were indistinguishable morphologically. Loss of the white V resulted in a phenotype which, as far as is known, does not occur naturally. Two such yellow-tip phenotypes were found (plate I, fig. 2) differing in intensity of the yellow coloration, and in the area of the yellow tip. The relative frequencies with which the loss phenotypes occurred as whole leaf mutations, summed over the three experiments, were :—

Loss of whole mark	103
Loss of white V	80
Loss of yellow tip	18

The implications of the relative frequencies of occurrence and nature of these mutant phenotypes, in relation to the structure and possible complexity of the V^{by} locus has already been discussed (Davies and Wall, 1959). The second class of mutants included some in which the white V had been reduced to spots and the yellow tip reduced in size (plate I, fig. 3) and others in which the whole mark had been reduced in intensity (plate I, fig. 4). Again these phenotypes are not known to occur naturally. Genetical analyses of the mutant phenotypes has not been possible as the mutants proved to be chimæric and the flowers derived from normal tissue.

8. DISCUSSION

It must be emphasised that a given radiation treatment in one of the experiments reported need not necessarily have the same percentage mutations recorded as the same treatment in another experiment. The reason for this is that from the time of appearance of the first mutation, there is a definite increase in the frequency up to a given time, after which the frequency decreases, while the number of leaves increases exponentially all the time. The total percentage mutations scored will depend on the stage in this cycle at which scoring is terminated.

Plate II

120 curie Co^{60} installation at the Wantage Radiation Laboratory.

Again the rate of development of the cycle is directly dependent on the environment. As scoring did not terminate at the same stage in all three experiments and as they were undertaken at different seasons a comparison of the percentage mutant leaves between experiments is not valid, but within any given experiment comparisons are accurate.

The response of this tissue to gamma radiation, in terms of induced mutations at the V^{by} locus has been shown to be dependent on a number of factors. The relation of frequency to dose fitted both a linear and quadratic function; the first part of the frequency dose response (up to 1500 rads) appeared to fit a quadratic more closely than a linear function. At higher doses this trend was not maintained, and the apparent overall agreement with linearity could have been due to an elimination, through dominant lethal aberrations, of the most badly damaged cells at the highest doses. Thus multi-hit events may have constituted a proportion of the events scored, and these would to some extent account for the reduction in the frequency of mutations recovered when a given dose was fractionated, or the dose rate was lowered. This cannot be the sole explanation for the reduction observed, as the value obtained after exposure to two equal fractions separated by two or four days usually did not approach twice that obtained with a dose equivalent to one fraction. Again the extent of the reduction due to a lowering of the dose rate was shown by the fact that 2400 rads given at a rate of 25 rads per hour produced a number of mutations similar to 600 rads given at a rate of 1100 rads per hour. It is known that ionising radiations affect cell development, inhibiting D.N.A. synthesis and retarding the development of the mitotic cycle, but even if the first fraction retarded or even inhibited division prior to and during exposure to the second fraction, it is unlikely that the variations in the sensitivity of different stages of dividing and resting cells could account entirely for the reduction observed on fractionating a given dose. Again Lane (1952) has suggested that one fraction may modify the sensitivity of a tissue to a later fraction but others have failed to confirm this result. A fuller elucidation of the fractionation effect must await further investigation.

The results of Russell *et al.* (1958, 1959) are of interest in connection with the effect of intensity on mutation rate. They have shown that the mutation rates of oocytes and spermatogonia, but not of spermatozoa, are dependent on dose rate, and have suggested that the differential response of these classes is related to the possibility of a repair of damaged sites at low dose rates in oocytes and spermatogonia but not in spermatozoa.

A genetic control of mutational response at a given locus has been clearly shown in the present experiments. Though the two clones utilised were derived from two full sib plants, as *Trifolium repens* is an obligate outbreeder the genetic constitution of the sibs could have differed to a marked extent. Several hypotheses can be proposed o account for the difference, but all lack experimental verification.

Differences could exist in the nature and amounts of protective or sensitising substances, in the respiration rates and oxygen contents, and in the metabolic rates of the tissues. Genetic control of spontaneous chromosome breakage is known to occur and in this instance a similar control of frequency or localisation of induced breaks or mutations could occur.

From a plant breeder's viewpoint these studies have many implications. The occasional desirability of separating two loci so closely linked that crossing-over either occurs extremely infrequently or not at all, has been emphasised (Frankel, 1958) and in the present experiment this was achieved. The new phenotypes produced may prove valuable in contributing to a solution of the difficulties of seed certification in clovers, where the problem of authenticity is complicated (Davies, 1957). A programme is now being undertaken in which seeds heterozygous for V^{by} and *v* are being irradiated in an attempt to produce mutants similar to those illustrated in plate I, figs. 2, 3 and 4 and which will not be chimæric. Again the information obtained from the comparisons of acute, chronic and fractionated doses has provided more critical data for determining the possible value of gamma fields as a tool for the plant breeder. In terms of the frequency of induction of mutations it is obvious that acute are more efficient than chronic treatments, and though it has been claimed that gamma fields are advantageous for treating large specimens such as fruit trees, it has yet to be demonstrated that the treatment of scions or cuttings with acute doses is in any way less efficient than the treatment of whole trees with chronic doses.

9. SUMMARY

1. Two clonal lines of *Trifolium repens* heterozygous for the leaf marking alleles V^{by} and *v* have been utilised in a study of induced somatic mutations at the V^{by} locus.

2. Data are given on the dose-response relationship, and on the comparative efficiencies of acute, fractionated and chronic doses of gamma radiation.

3. Fractionated and chronic doses have in all instances given considerably lower frequencies of mutations than acute doses. This can in part be attributed to the multi-hit nature of some of the events scored, to an increase in the proportion of resting and hence more resistant cells in tissues exposed to an initial fraction of radiation, and to a changed sensitivity of irradiated cells.

4. A pronounced effect of genotype on the mutagenic sensitivity of the V^{by} locus was demonstrated.

5. Two elements have been separated at the V^{by} locus, which as far as is known are not separable by crossing-over, and mutant phenotypes produced which do not occur naturally.

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DEVELOPMENTAL VARIATION IN CHROMOSOME BEHAVIOUR

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1. INTRODUCTION

CHANGES which initiate anatomical and physiological differentiation during development also affect chromosomes, whose form and behaviour in consequence may vary between cells of different tissues. A familiar and extreme example is the difference between the chromosomes of the salivary gland cells and those, a hundred times smaller, of nerve ganglion cells in *Drosophila* larvæ. Differences may sometimes be detected also between component cells within tissues. Thus in the nurse cells of *Drosophila* ovaries a proportion of cell nuclei are polyploid, the remainder diploid (Hertwig, 1935). Chromosome variation therefore provides an additional and useful means for investigating the patterns of cell variation arising during development, especially within tissues where the differences in cell anatomy and physiological activity are otherwise difficult or impossible to detect. In the following account two characters, chiasma frequency and the timing sequence of cell divisions, are investigated at meiosis in the pollen mother cell (p.m.c.) tissue of rye anthers. The aim was, first, to determine whether definite patterns of variation for the characters could be distinguished within the anthers and, second, to inquire into the relations between them.

2. CHIASMA FREQUENCY

(i) *Method*

To determine the variation along its length each anther was cut transversely into four equal sections—numbered 1 to 4 from base to tip. Where metaphases were available in all sections the chiasma frequency was then scored from a sample of cells in each. The plants used were from five inbred, homozygous, lines (see previous papers, e.g. Rees, 1955) and F_1 hybrids between them.

(ii) *The results*

Table 1 gives the chiasma frequencies per section in one anther from each of 14 inbred plants and 12 F_1 hybrids. The results for each genotype are represented also in fig. 1.

In this graph a definite pattern of variation within the anther is indicated, the chiasma frequency tending to decrease from one end to the other.

A more detailed analysis of the data was desirable and this was achieved by fitting polynomial regressions. In the analysis of variance

TABLE 1

Chiasma frequencies per section in rye anthers. Twenty cells were scored in each section

Plant	Section			
	1	2	3	4
$P1^1$	253	252	252	251
$P1^2$	239	248	247	250
$P6^1$	239	250	241	244
$P6^2$	252	236	246	237
$P6^3$	255	257	246	254
$P7^1$	252	240	231	249
$P7^2$	244	244	241	229
$P7^3$	251	245	235	250
$P12^1$	242	247	245	240
$P12^2$	252	240	238	240
$P12^3$	255	252	237	259
$P17^1$	249	247	238	242
$P17^2$	230		244	236
$P17^3$	247	246	237	231
1×6^1	287	282	281	275
1×6^2	285	285	283	281
1×6^3	280	275	278	279
1×6^4	283	277	278	284
1×6^5	285	282	280	278
1×6^6	271	273	275	267
1×7^1	283	282	282	279
1×7^2	281	282	279	281
1×7^3	286	282	280	277
1×7^4	282	269	273	269
1×7^5	274	280	271	269
1×7^6	276	276	277	277

of the regressions, which is given in table 2, the degrees of freedom for the linear, quadratic and cubic regressions were split into components depending on heterogeneity between genotypes and between plants within genotypes. The conclusions may be summarised as follows :—

1. There is a significant difference between the mean chiasma frequencies of genotypes—due, chiefly, to the heterosis for this character displayed by the F_1.

2. The significant mean linear regression confirms the gradient in chiasma frequencies from the base to the tip of rye anthers. A relation is clearly established between the chiasma frequency of p.m.c. and their location within the anther.

3. There is no significant heterogeneity for the linear regression between inbred or outbred genotypes. Within genotypes however it will be observed that the heterogeneity between plants is significantly

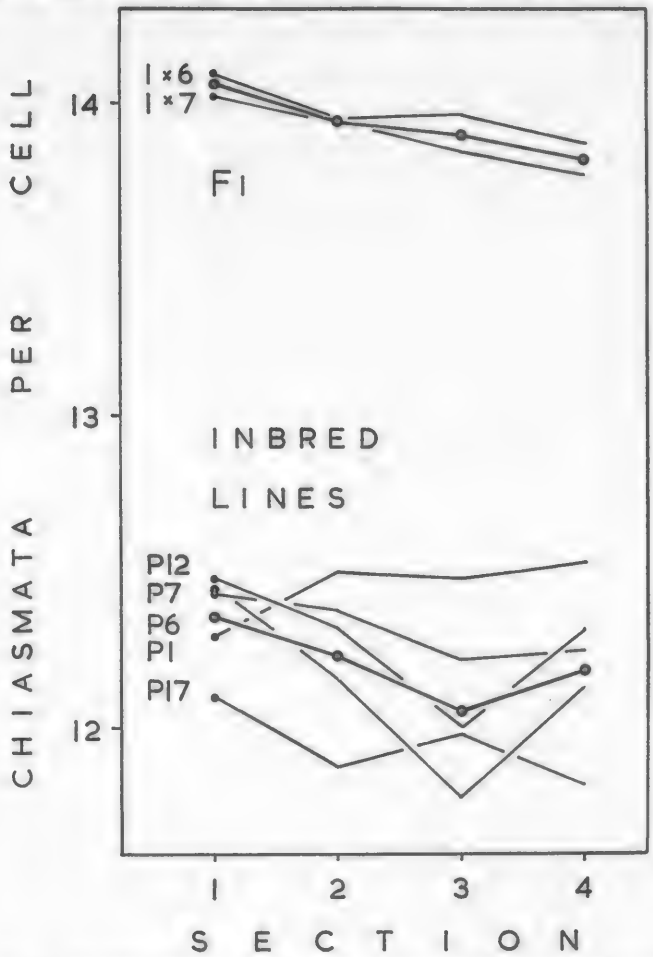

FIG. 1.—The distributions of chiasma frequencies in p.m.c. in sections of rye anthers.

larger for inbred than for F_1 families. Since plants within families are genetically identical it must be concluded that the pattern of developmental variation in the inbred, homozygous, individuals is less uniform, less consistent, than in the heterozygotes (cf. Rees and Thompson, 1956).

4. Neither the mean quadratic nor cubic regressions are significant.

It would appear however from fig. 1, particularly in respect of inbred lines, that there is variation in chiasma frequency distribution that is independent of and additional to that accounted for by the linear regression. Thus section three in anthers of inbred plants tends to have disproportionately low chiasma frequencies.

Further evidence for this additional variation comes from the analysis itself. The mean squares for heterogeneity within inbred and F_1 families for both the quadratic and cubic components are significantly

TABLE 2

Polynomial regression analysis of variance—chiasma frequency against anther sections

Item		N	MS		VR	*P*
Linear	Joint regression	1	264·69		10·61	0·001-0·01
	Het. between inbreds and F_1	1	0·47			
	Het. between inbred genotypes	4	23·46			
	Het. between F_1 genotypes	1	1·50	24·94		
	Het. within inbred genotypes	9	45·74			
	Het. within F_1 genotypes	10	11·60			
Quadratic	Joint regression	1	60·01		2·103	0·10-0·20
	Het. between inbreds and F_1	1	33·58			
	Het. between inbred genotypes	4	27·90			
	Het. between F_1 genotypes	1	0·52	28·53		
	Het. within inbred genotypes	9	51·09			
	Het. within F_1 genotypes	10	10·77			
Cubic	Joint regression	1	7·63			
	Het. between inbreds and F_1	1	29·82			
	Het. between inbred genotypes	4	17·69			
	Het. between F_1 genotypes	1	3·04	28·82		
	Het. within inbred genotypes	9	61·02			
	Het. within F_1 genotypes	10	6·78			
Mean chiasmata	Between inbreds and F_1	1	30,556·73			
	Between inbred genotypes	4	158·14			
	Between F_1 inbreds	1	28·52	117·25		
	Within inbred genotypes	9	93·07			
	Within F_1 genotypes	10	65·75			

different. They are larger between inbred than F_1 plants. This variation within inbred families also, of course, confirms the earlier conclusion of the developmental instability of the homozygotes as compared with the heterozygotes.

Having established a definite distribution pattern for chiasma frequency within anthers it is worthwhile now inquiring, as far as possible, how, during development, it originates. In *Allium* Darlington and Haque (1955) have demonstrated that localised abnormal chromosome behaviour, including failure of metaphase pairing, is associated with abnormal *dyschronisation* in the division of p.m.c. In view of this it was worthwhile inquiring whether "normal" differences in the timing of cell divisions can be correlated with the developmental variation in chiasma frequency. In the next section therefore, data are considered with regard to the timing of cell divisions in the four sections of the anther.

3. THE TIMING OF MEIOSIS

To begin with the problem was to determine whether or not divisions in the four sections were synchronised. Data for this purpose were available for six anthers of a *P*12 plant (*P*12[4]) scored in 1954. The p.m.c. of each section were classified according to their stage of

TABLE 3

The distribution of meiotic stages in anther sections of P12[4]

Anther	Section	No. of p.m.c.			"M.I." (in angles)
		At stages earlier than metaphase	At metaphase	Later than metaphase	
1	1	0	35	19	56
	2	0	7	48	76
	3	0	14	42	70
	4	1	57	12	50
2	1	0	50	0	50
	2	0	28	48	65
	3	0	50	4	47
	4	7	42	2	42
3	1	1	48	1	45
	2	0	54	10	50
	3	10	56	0	41
	4	19	49	0	37
4	1	1	74	0	45
	2	0	74	1	46
	3	6	64	0	43
	4	33	20	0	26
5	1	0	30	0	44
	2	0	34	0	44
	3	3	28	0	42
	4	19	20	0	31
6	1	0	20	26	63
	2	0	11	37	71
	3	0	25	31	61
	4	3	24	30	60

meiosis. The results for three classes of cells, (1) those at first metaphase, (2) at stages earlier and (3) at stages later than first metaphase are given in table 3.

Chiasma frequencies also were scored in these anthers and are given in table 4.

For analysis of the timing data a time scale, the *metaphase index* (M.I.), was calculated as follows :—

$$\frac{100-a+b}{2}$$

where a is the percentage of cells at stages earlier than first metaphase and b is the percentage of cells at stages later than first metaphase.

Because they are based on percentages the M.I. estimates were subsequently transformed to angular values for statistical analysis, and these angular values are also presented in table 3. Sections with low

TABLE 4

Chiasma frequencies per section in anthers of P12[4].
Ten cells were scored in each section

Anther	Section				Total chiasmata
	1	2	3	4	
1	121	115	110	116	462
2	113	115	120	115	461
3	115	116	117	116	464
4	122	121	113	117	473
5	119	115	113	120	467
6	118	118	109	116	461
Total	708	700	682	700	

M.I.s, where cells at first metaphase will be amongst the first to reach that stage, will be less advanced in division than those with high M.I.s, where most cells will have already progressed beyond metaphase. It

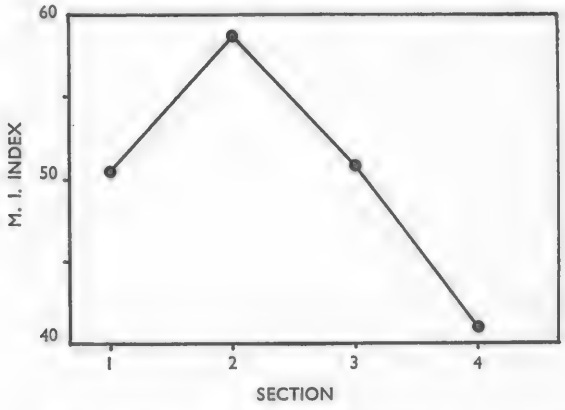

FIG. 2.—The M.I. distribution in anther sections of *P12*[4] (see text).

must, of course, be borne in mind that a difference in M.I. does not necessarily imply that p.m.c. proceed through prophase to metaphase at different rates; rather that they may do so at different times.

By means of this arbitrary time scale we can now inquire in some detail into the sequence of meiotic divisions in different regions of the

anther. It will be immediately apparent from the table that timing is variable between sections. It will be seen furthermore that the variation in timing is consistent between anthers; 2 is the most advanced, 4 the least and 1 and 3 are intermediate. The general pattern is illustrated in fig. 2.

TABLE 5

Polynomial regression analysis—M.I. against anther sections

Item		N	MS	VR	*P*
Between anthers in mean M.I.		5	479·54		
Linear	Joint regression	1	399·68	37·11	0·01
	Het. between anthers	5	10·77		
Quadratic	Joint regression	1	477·04		
	Het. between anthers	5	34·94	13·65	0·05-0·01
Cubic	Joint regression	1	63·08	2·82	
	Het. between anthers	5	22·37		

A statistical analysis, by means of polynomial regressions as for chiasma frequency, shows that the linear and quadratic components are significant whereas the cubic regression mean square is not (table 5). The analysis confirms the relation between the timing of meiosis in p.m.c. and their distribution. It shows too that while the basal region is in general more advanced than the tip the timing gradient is not a simple, that is a linear, function of distance—as indeed the graph indicates.

One notes in relation to the above that the vascular system of the anther (fig. 3) is such that the probable order of supply of nutrients and other substances to the sections is 2, 1 and 3, 4—the order of increasing distance from the point of entry of the vascular bundle. It is not unreasonable to suppose therefore that stimulation causing the onset of meiosis, being in the same section order, is provided by a substance diffusing inwards along the vascular strand. Such a substance would not necessarily act immediately but perhaps, through the medium of the cytoplasm, over a period of one or more cell generations.

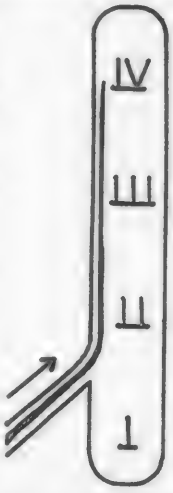

FIG. 3.—The vascular supply to a rye anther.

It has now been established that both in the timing of their meiosis and in the frequency of chiasmata which they form during meiosis the p.m.c. vary in quite well defined developmental patterns within the anthers. In the following section we shall consider whether there is evidence for a causal relation between the two.

4. TIMING AND CHIASMA FREQUENCY

The average chiasma frequency distribution in *P*12[4] (see fig. 4) is in good agreement with the 1958 data for inbred lines generally and for

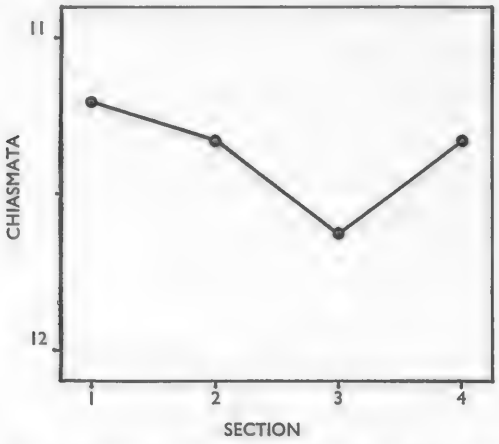

FIG. 4.—Chiasma frequencies per cell in sections of *P*12[4] anthers.

*P*12 in 1958 especially. We note in particular the corresponding low values in section 3.

The relation between chiasma frequencies and M.I. in *P*12[4] has been investigated at two levels, the anther and the sections. The results were as follows.

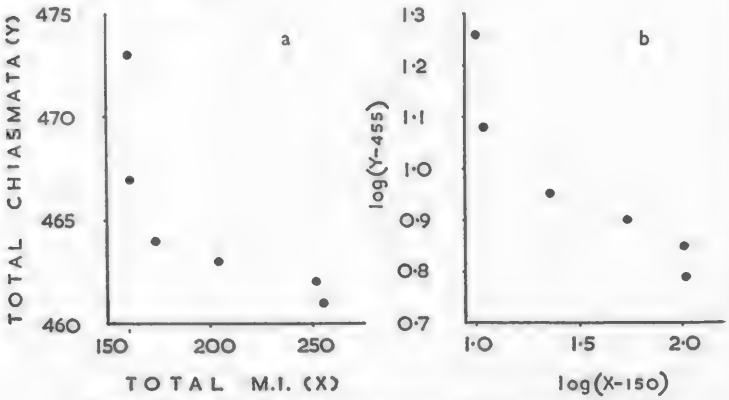

FIG. 5.—Chiasma frequencies plotted against M.I.s of anthers of *P*12[4].

Anthers. In graph 5*a* the total chiasmata scored in each *P*12[4] anther is plotted against the total M.I. of each. Transformation of the data with the aid of *logs* straightens this curve (5*b*) and permits a

simple test of significance by means of a linear regression analysis of variance. The regression is significant at the 0·01 level. Metaphases in the more advanced anthers, it is concluded, have lower chiasma frequencies than in the less advanced. Put in another way, the chiasma frequencies are higher in p.m.c. which come into metaphase first.

The correlation between timing and chiasma frequency is, of course, exactly what would be expected on the basis of the precocity theory, (Darlington, 1940). While compatible with this theory the evidence however is not entirely conclusive because, as mentioned earlier, it is not known whether the chromosomes in p.m.c. at metaphase at different times proceed through meiosis, particularly chromosome pairing, at different rates.

TABLE 6

Analysis of variance of mean chiasma frequencies in $P12^4$ (*see text*).

Item	SS	N	MS
Between anthers .	24·50	5	4·9
Between sections .	60·50	3	20·16
Residual . .	167·50	15	11·16
Total .	252·50	23	

Sections. If p.m.c. dividing early have higher chiasma frequencies than those dividing later it might be expected that sections with low M.I.s, with the first crop of p.m.c. at metaphase, would have higher chiasma frequencies than those with high M.I.s.—where p.m.c. at metaphase would represent the late dividing cells in that region. While such a relation is not ruled out it is clear from a comparison of graphs 3 and 4 that the chiasma frequency variation between sections within an anther is by no means directly or completely dependent on timing. In these graphs it will be seen that the high and low $P12^4$ chiasma frequencies do not correspond with the low and high M.I.s for sections. Another cause of chiasma frequency variation between sections is indicated.

A clue to one additional factor controlling the chiasma frequency variation between sections is provided by a straightforward analysis of variance of the chiasma frequencies in $P12^4$ (table 6). It will be seen that the mean square for variation between sections is 4·1 times greater than the mean square for variation between anthers. While not significant at the 5 per cent. level (P=0·1-0·05) the much smaller variation between anthers, as compared with that within, suggests a limit to the number of chiasmata per anther and, further, the possibility of competition for chiasmata between sections. Competition for chiasmata within cells is, of course, well established by earlier work (e.g. Mather, 1936).

Evidence confirming and at the same time indicating the nature of the competition between sections is provided by further comparisons between timing and chiasma frequency within anther sections. In fig. 6 section chiasma frequencies are plotted against section M.I.s for

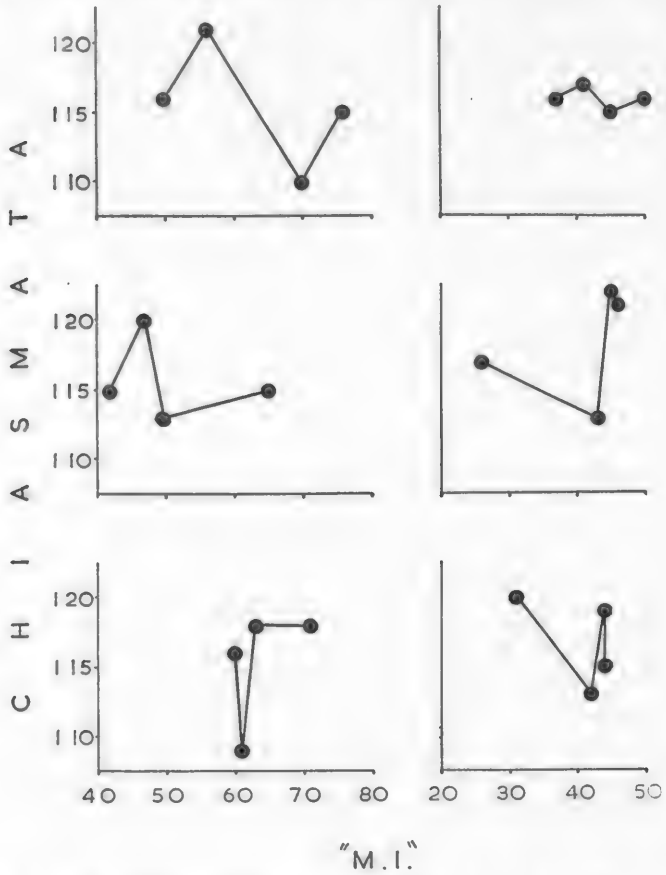

FIG. 6.—Section chiasma frequencies plotted against section M.I.s in six anthers of P12[4]. Note alternation of chiasma frequencies in relation to timing.

each anther separately. It will be observed that the chiasmata/M.I. curves are characteristically "zig-zag". The chiasma frequencies alternate or, rather, are compensating between successive sections. The compensation it will be observed relates to the distribution of sections with respect to their timing succession, not directly to their spatial distribution. Thus when the chiasma frequencies of the first two sections to divide are high and low respectively that of the third

will be high, the fourth low ; or alternatively the sequence may be low, high, low, high.

It will be recalled that substances were inferred to diffuse into the anther in order of sections 2, 1 and 3, 4. Given that a substance limiting chiasma frequency and itself of limited amount is supplied to sections in this order the compensation effect can be explained where excessive absorption by one region curtails the supply to the next and vice versa. Whether timing and chiasma frequency are influenced by the same substance or by different substances acting independently cannot be established by the present data.

5. SUMMARY AND CONCLUSIONS

Differences in chromosome behaviour between cells are shown to be a useful index of the developmental variation occurring within a tissue. For two characters investigated in rye p.m.c., namely chiasma frequency and the timing sequence of meiotic divisions, well defined patterns of variation are demonstrated within anthers. Variation in both characters is probably influenced by the system of distribution of nutrient or other substances to the anther during its development.

The pattern of developmental variation in chiasma frequency is more consistent in anthers of heterozygous than homozygous plants.

Timing and chiasma frequency are related ; p.m.c. reaching metaphase early have higher chiasma frequencies than those at metaphase later.

Another factor affecting chiasma frequency variation within an anther is competition for chiasmata between different regions.

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HAPLOID INTERSEXES IN THE WASP *HABROBRACON*

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1. INTRODUCTION

A locus responsible for sex determination is known in the parasitic wasp *Habrobracon*. Whiting (1943*a*) described nine alleles at this locus, any of which in pairwise combination produced diploid females, any of which when homozygous produced diploid males, and any of which when hemizygous in unfertilised eggs produced haploid males. It follows that half of the haploid males in a given stock are genotypically different from the other half with respect to sex alleles, although they are phenotypically indistinguishable.

Three different classes of sex intergrades have been observed in *Habrobracon*: *gynandromorphs*, individuals that are a composite of true haploid male and diploid female parts; *gynandroids*, haploid males mosaic for haploid nuclei of two different sex alleles; and *intersexes*, individuals whose cells and tissues are of one chromosomal constitution yet which show feminisation of male tissue or masculinisation of female tissue.

Gynandromorphs usually come from eggs in which two meiotic products become pronuclei, one of them combining with a sperm and the other entering cleavage "asyngamate". They can, however, come from unfertilised eggs when two of the four meiotic products fuse and enter cleavage and either one or both of the remainder enter cleavage without fusing. They also can come from fertilisation of the egg with two sperms, one of which combines with the egg pronucleus and the other enters cleavage asyngamate. (See von Borstel, 1957, for a review of the multiple causes of gynandromorphism.)

The gynandroids described by Whiting, Greb, and Speicher (1934) were understood genetically when the nature of sex allelism was established. At the boundaries between haploid male tissues of different sex allele composition, female tissue was formed, presumably by diffusion and complementation of agents produced by cells containing the different alleles. This phenomenon has now been recognised as operating in enzyme synthesis in *Neurospora* (Woodward, Partridge, and Giles, 1958).

The intersexes seem to have two different genetic derivations, each of which has been explained by assuming that mutations or chromosomal rearrangements have occurred at the sex locus. Whiting (1946) described a diploid intersex—a masculinised female. To explain it, he proposed that a mutation of one of the sex alleles had occurred so

* Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

that complementation was imperfect. A haploid intersex, found twice previously (Whiting, 1943*b* ; Whiting and Starrells, 1950) was obtained under conditions that allowed a partial analysis of its composition. Whiting and Starrells postulated that the intersexes may have arisen from a translocation involving the sex locus with a consequent separation of the sex alleles. This would result in establishment of the two complementary alleles in one genome and thus produce haploids with both sex alleles in every somatic nucleus. In their argument diploidy became a further essential factor for femaleness because haploid intersexes rather than haploid females were produced. We discovered the intersex mutants during a routine test for translocations, so that the hypothesis of Whiting and Starrells could be tested. In the first two occurrences of these intersexes the wasp females that produced the offspring segregating into two classes (males and sex intergrades) died before their condition was discovered and therefore before they were mated. Unfortunately this again was the case.

2. EXPERIMENTAL

The mutant female that produced the intergrades occurred in an experiment designed to measure the frequency of inherited partial sterility (probably translocations) in sperm after 4000 r.

When the F_1 females from irradiated $\neq 17o^i$ fathers crossed with $\neq 33$ wild type virgins were bred unmated to determine possible heterozygosity for mutations, one of the virgin females had 11 normal sons and 13 sex intergrades that resembled gynandromorphs since they had male heads and female abdomens. The wing-cell size and antennal length of these intergrades appeared to be typically male, and the behaviour of the wasp was distinctly male. They differed from gynandromorphs, however, in that the ovaries of the intergrades were saclike and undeveloped. It can be concluded, therefore, that these are true intersexes. The femaleness-to-maleness gradation from posterior to anterior of these individuals was in accord with the description of Whiting (1943*b*).

The locus responsible for intersex production exhibited linkage with the orange locus (chromosome 2). Ten males were wild type and one was ivory eyed (an allele at the orange locus) ; three of the intersexes were wild type and ten were ivory eyed (table 1). This mutation had occurred twice previously and both times the genetic construction of the cross was such that linkage to the same locus was detected. The first occurrence (Whiting, 1943*b*) was spontaneous in a cross of wild type with orange, and the second (Whiting and Starrells, 1950) was induced by X-rays in a wild type female subsequently crossed with a male bearing the ivory allele of the orange locus. The linkage data from the three occurrences were analysed by coincidence χ^2 to estimate the probability that each occurrence was at the same locus. For this, $P=0{\cdot}50$ and it is assumed the mutations are at the same position. Of

the 69 intersexes, 13 showed crossing-over ; and of the 63 males, 21 were cross-overs. Thus, 34 of 132 individuals were cross-overs. All the data indicate that the linkage of the intersex mutation to the orange locus is 0·26.

Hatchability and adult survival for the mother of the intersexes are shown in table 2. The wasps were raised in four broods. The first brood of 11 eggs (producing 11 adults) was discarded before the

TABLE 1

Distribution of eye colours among intersexes and males in offspring of each occurrence of the intersex mutation

	Whiting (1943*b*)		Whiting and Starrells (1950)		Present occurrence		Total
	Black	Orange	Black	Ivory	Black	Ivory	
Intersexes	17	5	29	5	3	10	69
Males .	7	17	13	15	10	1	63

intersex nature of some of the offspring was recognised. These 11 are not included in table 1. The reduction in adult survival to 0·81 is not consistent with the assumption that the female was heterozygous for a recessive lethal since adult survival in controls is normally about

TABLE 2

Hatchability and adult survival of eggs laid by mother of intersexes

Viable eggs/total	Hatchability per cent.	Viable adults/total eggs	Adult survival per cent.
43/43	1·00	35/43	0·81

0·85-0·90, and with a recessive lethal mutation adult survival should have been less than 0·50. The deaths are presumed to have been accidental in this case (and in controls for that matter) since 7 of the 8 deaths were in one brood of 15 eggs. The lethality occurred during early larval and mid-pupal development, which is a common time of death when too many larvæ are crowded on one host caterpillar (10 to 12 larvæ per caterpillar is about maximum to ensure total survival). Six F_2 brothers of the intersexes were outcrossed to wild type females to determine whether some of the F_2 sons were heterozygous and the intersex condition was heritable through the normal-appearing males. The F_3 daughters all had the expected normal hatchability and adult survival of offspring. All F_4 adults were normal.

3. DISCUSSION

The exact nature of these sex intergrades is not immediately obvious; consequently, two different hypotheses will be examined : (1) a translocation was induced involving the sex alleles, and (2) a mutation was induced that involved a modifier locus analogous in some respects to the transformer or intersex locus in *Drosophila*.

Whiting and Starrells (1950) proposed that a translocation could involve the sex locus (chromosome 1) and the orange locus (chromosome 2). Under these conditions, the fertilised egg would become a normal female heterozygous for the translocation, and, accordingly, her offspring would be of four classes : (1) normal males, (2) males bearing the translocation, (3) haploid intersexes bearing both sex alleles, and (4) embryos bearing no sex alleles and presumed to be inviable.

Such a translocation would have to be very small, perhaps a transposition of the sex locus only, since classes (3) and (4) would be of the adjacent I segregation type, and these are invariably lethal through chromosome imbalance of the duplication-deficiency type when large pieces of the chromosomes are translocated.

There are several objections to the translocation hypothesis. First, if it had been a transposition in all three recorded cases, then it would have been unlikely that a small transposition of the sex alleles from chromosome 1 to the same position on chromosome 2 would have occurred three times in succession. Second, one-third of the viable offspring from a heterozygous female should be intersexes rather than one-half (table 1) if the alternate and adjacent I segregation classes are equal. Third, the class (2) males should be viable and, when mated, should produce females heterozygous for the translocation ; no such translocations appeared in the last recurrence of the haploid intersexes either as inherited partial sterility or production of more intersexes. Fourth, the hatchability, and probably the adult survival, of eggs from the F_1 virgins following radiation (table 2) is too high to admit the possible existence of any inviable class.

The second hypothesis, that a mutation was induced involving a modifier locus analogous in some respects to the transformer or intersex locus in *Drosophila* (the locus is 26 units from the orange locus on chromosome 2), is consistent with the known facts and is most easily reconcilable with the observation that each occurrence of the intergrade mutation has been in the same position on the chromosome.

The precedent for a mutation of this type is seen in the following mutations : *ix* and *tom* (renamed ix^2, Meyer, 1958) on the second chromosome and *Hr* and *tra* on the third chromosome of *Drosophila melanogaster* ; Ix^B on the second chromosome and Lebedeff's recessive on the third chromosome of *D. virilis* ; Sturtevant's recessive intersex mutation in *D. simulans* ; and Dobzhansky and Spassky's dominant intersex in *D. pseudoobscura* (reviewed in part by Kroeger, 1959, and

in part by Gowen and Fung, 1957). In every case genetic females are transformed toward or into phenotypic males. In the wasps *Mormoniella* (Saul, 1960) and *Habrobracon*, however, genetic males are transformed toward phenotypic females by the presumed intersex gene. As in all the transformed or intersexual *Drosophila* and the intersexual *Habrobracon*, the gonads of *Mormoniella* are reduced to almost vestigial structures. The one case in *Habrobracon* for a female-to-male intersex should not be considered in this context since, according to Whiting (1946), it is possible that the sex allele itself had been mutated. The only suggested case that does not follow the general rule for a female-to-male sex shift in *Drosophila* was that of the *Bd-M* combination (Goldschmidt, 1947, 1949), but this was disputed by Sturtevant (1949) on the grounds that these individuals merely had underdeveloped male genitalia. Kroeger (1960) based a strong argument for Sturtevant's contention on the finding that, although the *Bd-M* combination produces only underdeveloped males, an environmental effect in conjunction with the *fa*^{no} mutation yields a graded series of underdeveloped, through normal, to overdeveloped male genitalia. Thus it seems that no clear cases for male-to-female intersexuality exist in *Drosophila*, whereas female-to-male intersexual mutations have appeared a number of times in several of the *Drosophila* species. It can be concluded that the relation between sex-modifying genes and the direction of change that they produce in the wasps is the reverse of that found in *Drosophila*.

Furthermore, in the *Habrobracon* intersexes, a female-to-male gradient exists from the posterior toward the anterior end ; whereas in *Drosophila ix* mutations, the reverse is observed (Kroeger, 1959). Another gene, gynoid (chromosome 1), that exhibits certain characteristics of intersexuality in *Habrobracon* by showing a weak male-toward-female transformation does not show this type of gradient. Gynoid males, although they have shortened antennæ and thickened abdominal tergites and sternites, have male ocelli and genitalia and exhibit normal mating behaviour and fertility (Whiting, Greb, and Speicher, 1934). If any morphological gradient exists at all in gynoid mutants, the tergites and sternites in the anterior of the abdomen look a little more female and grade to a more male appearance toward the posterior, thus being the reverse of the other *Habrobracon* intersex mutants and indicating that any generalisations based on a posterior-to-anterior gradient may be spurious.

The question arises as to the relation of intersexuality genes to sex determination. This has been discussed at length elsewhere (see for instance, Gowen and Fung, 1957, and Goldschmidt, 1955, pp. 90-94 and 448-451) and is beyond the scope of this paper. It is possible that the intersex gene in *Habrobracon* suppresses or releases to a certain degree the action or complementary activity of the sex locus. Final resolution of this problem will probably have to await elaboration of the biochemical basis of sex determination.

4. SUMMARY

During a routine analysis for radiation-induced recessive lethal mutations and translocations, a female was found that produced haploid intersex offspring. These intersexes showed linkage with the orange locus of chromosome 2, as had two previous occurrences of this type of intersex. The intersexes were shown not to have derived from a separation of the sex alleles by a translocation. It is suggested that a gene was mutated that is in some respects analogous to the genes for intersexuality found in different species of *Drosophila*. The action in *Habrobracon* would appear to be from male-toward-female rather than the reverse as in *Drosophila*.

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AN INTERRACIAL HYBRIDISATION EXPERIMENT IN NATURAL POPULATIONS OF *DROSOPHILA WILLISTONI*

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As many as 66 different inversions evenly distributed in the five chromosomal arms are known in *Drosophila willistoni*, the most variable species of this genus so far studied in regard to this characteristic. Nevertheless, their frequency in natural populations varies greatly. In marginal populations, studied by Townsend (1952), da Cunha, Burla and Dobzhansky (1950), Dobzhansky (1957) and Cordeiro (1959), the X chromosomes are almost completely free of inversions. Also, the average number of inversions per individual decreases from the centre toward the edges of the species distribution. Interesting questions can be raised in connection with these findings. For instance, are these marginal populations able to accept heterotic gene arrangements of different origins? What will be the reaction of such populations to new gene orders from central populations?

Another aspect of interest is that Dobzhansky (1950), Dobzhansky and Pavlovsky (1953), and others, clearly showed that the equilibrium frequencies of heterozygous inversions are different if intra- or inter-geographic population hybrids are used. The experimental study of inversion properties, on the other hand, was almost always made in artificial populations. Will the same principles apply to experiments performed under natural conditions?

This paper describes the results of cytologic analysis made during 1956 and 1957 of inversion frequencies in four semi-isolated natural populations of *Drosophila willistoni*. In three of them hybrids between populations of different geographic origin have been introduced.

1. MATERIAL AND METHODS

Some regions of Rio Grande do Sul, the southernmost State of Brazil, are covered by grassland, with scattered small woods isolated from each other by varying distances of hundreds of metres to several kilometres. *Drosophila willistoni* does not live in the grassland, building its semi-isolated populations in these true islands of woods, the "capões". This is the situation near the locality of Tainhas, in the Plateau of São Francisco de Paula's District, the place chosen for the experiment now reported.

Samples of more than one hundred individuals from Feliz, a locality near Tainhas, were crossed with northern Brazilian races, and the F_1 hybrids (North/South) cytologically analysed showed a high frequency of heterozygotes for inversions not observed in the natural populations of Feliz or Tainhas (table 1). A great number of F_1 (N/S) individuals was raised by daily transfer of 50 pairs in 1/4 litre bottles with banana-agar-yeasted food. To avoid competition, extra amounts of yeast were distributed two days after the transfers. By counting five samples of 1000 individuals, an average weight was established for these F_1 (N/S) and, with the aid

TABLE 1

Frequencies in per cent. of heterozygotes for inversions in populations of Drosophila willistoni *from experimental woods*

Chromosome arm and inversion	Control Wood III		Wood I				Stock released in woods IV and V 1956	Wood IV		Wood V	
	1956	1957	Stock released 1956	Sample 1956	Sample 1, 1957	Sample 2, 1957		Sample 1, 1956	Sample 2, 1956	Sample 1, 1957	Sample 2, 1957
XL None	96·9	100·0	—	76·8	92·3	95·6	—	49·4	100·0	100·0	98·6
A	3·1	—	—	2·4	2·6	—	—	1·1	—	—	1·4
B	—	—	—	3·7	—	—	—	—	—	—	—
B_1	—	—	—	—	2·6	—	54·5	29·2	—	—	—
D	—	—	100·0	17·1	2·6	2·2	95·4	47·2	—	—	—
E_1	—	—	—	—	—	—	—	2·2	—	—	—
F	—	—	—	—	—	2·2	77·3	29·2	—	—	—
G	—	—	—	—	—	—	77·3	29·2	—	—	—
H	—	—	—	—	—	—	4·5	—	—	—	—
XR None	96·9	100·0	—	80·5	94·9	91·3	—	49·4	100·0	100·0	93·0
A	—	—	—	—	5·1	—	—	—	—	—	7·0
B	—	—	—	—	—	4·3	—	—	—	—	—
C	3·1	—	—	—	—	2·2	72·7	31·5	—	—	1·4
D	3·1	—	—	—	—	—	72·7	31·5	—	—	—
E	—	—	100·0	19·5	—	2·2	27·3	27·0	—	—	—
F	—	—	—	—	—	—	—	1·1	—	—	—
Females studied	64	58	24	82	39	46	22	89	12	31	71
IIL None	22·1	29·2	3·1	22·2	34·0	34·5	28·1	30·8	35·3	17·9	32·9
A	15·8	21·5	81·2	5·6	5·7	10·9	3·1	—	—	2·6	18·8
B	20·0	9·2	6·2	33·3	24·5	10·9	12·5	25·6	11·8	28·2	12·9
B_1	—	—	—	—	—	—	—	1·7	—	—	—
C	1·1	1·5	18·7	0·9	5·7	3·6	6·2	1·7	—	5·1	7·1
D	45·3	27·7	31·2	43·5	45·3	36·4	34·4	35·9	35·3	51·3	34·1
E	45·3	36·9	37·5	43·5	47·2	36·4	37·5	35·9	35·3	48·7	37·6
F	23·2	33·8	34·4	28·7	30·2	27·3	31·2	24·8	23·5	35·9	31·8
G	—	—	—	0·9	—	—	—	—	—	—	—
H	18·9	3·1	25·0	18·5	3·8	5·4	18·7	6·0	11·8	—	5·9
I	—	—	—	—	—	1·8	—	—	—	—	—

TABLE 1—*continued*

TABLE 1—*continued*

Chromosome arm and inversion	Control Wood III		Wood I				Stock released in woods IV and V 1956	Wood IV		Wood V	
	1956	1957	Stock released 1956	Sample 1956	Sample 1, 1957	Sample 2, 1957		Sample 1, 1956	Sample 2, 1956	Sample 1, 1957	Sample 2, 1957
IIR none	92·6	73·8	9·4	77·8	71·7	74·5	40·6	68·4	100·0	79·5	69·4
A	—	4·6	—	—	7·5	3·6	—	—	—	—	4·7
B	2·1	4·6	—	0·9	—	5·4	—	—	—	—	4·7
C	—	7·7	—	—	15·1	7·3	—	—	—	12·8	11·8
D	1·1	6·1	—	—	—	1·8	—	—	—	2·6	3·5
E	4·2	9·2	90·6	24·1	9·4	10·9	65·6	31·6	—	5·1	11·8
III None	20·0	36·9	3·1	12·0	17·0	23·6	9·4	21·4	17·6	33·3	25·9
A	16·8	10·8	18·7	—	—	7·3	62·5	—	—	5·1	7·1
B	48·4	21·5	28·1	49·1	47·2	32·7	31·2	40·2	58·8	41·0	38·8
B_1	—	1·5	—	—	—	3·6	—	—	—	—	4·7
C	15·8	15·4	34·4	37·0	11·3	14·5	—	38·5	35·3	15·4	17·6
D	1·0	3·1	3·1	—	—	3·6	—	—	—	—	—
E	—	—	—	—	—	3·6	—	—	—	—	—
F	1·0	1·5	50·0	14·8	1·9	—	—	1·7	—	—	—
H	—	1·5	—	—	—	1·8	—	—	—	—	—
I_1	1·0	—	—	—	—	—	—	—	—	—	—
J	34·7	41·5	78·1	47·2	52·8	47·3	56·2	42·7	35·3	38·5	38·8
L	—	—	—	—	—	—	—	—	—	2·6	1·2
M	—	1·5	—	—	—	—	—	—	—	—	—
N	—	1·5	—	—	—	—	—	—	—	—	—
P_1	—	—	—	1·8	—	—	—	—	—	—	—
Flies studied	95	65	32	108	53	55	32	117	17	39	85
Mean number of inversions per individual ♀	3·06 ±0·18	2·64 ±0·21	7·50 ±0·31	3·93 ±0·20	3·36 ±0·31	2·98 ±0·29	8·36 ±0·41	4·94 ±0·34	2·00 ±0·37	3·16 ±0·29	3·11 ±0·22
Mean number of inversions per individual ♂	2·9 ±0·23	2·86 ±0·62	5·00 ±0·45	3·50 ±0·28	2·64 ±0·55	2·55 ±0·52	3·30 ±0·43	3·53 ±0·38	3·60 ±0·61	2·12 ±0·41	2·71 ±0·36

of an analytical balance, the mass of F_1 (N/S) hybrid weighed was estimated to have contained about 180,000 individuals. Three isolated woods I, IV and V, previously chosen at Tainhas, received approximately 60,000 F_1 individuals each. The approximate distance between the control wood (III) and wood I is 7 km. ; between III and IV, 3 km. ; and between IV and V, 9 km. Wood I is also isolated from the

TABLE 2

Significant differences between inversion frequencies of control samples of D. willistoni

Chromosomes and inversions	Frequencies (per cent.)		χ^2	P (1 d.f.)
	1956	1957		
IIL-D	45·3	27·7	5·05	<0·05
IIL-H	18·9	3·1	8·88	<0·01
III-B	48·4	21·5	11·90	<0·001

others by the Tainhas river. Near wood I (1 km. away) there is wood II, not studied in detail, but considered initially as a control to detect migration of the F_1 hybrid N/S descendants.

Due to the cold winters, collections of *Drosophila willistoni* have been made in the summer seasons of 1956 and 1957 only. Cytologic analysis of the salivary glands were performed on single F_1 larvæ, produced by isolated cultures of one female each. The usual aceto-orcein technique was employed.

2. THE ISOLATION OF THE POPULATIONS

Some evidence may be given on the relative isolation of the populations studied. The F_1 hybrids were introduced in wood I at 10 a.m. on February 6, 1956. The females were heterozygous for the recessive sex-linked mutant *scute* ; half of the males were normal, and half carried *scute* as a marker. Intensive collections were made in wood II during all this day and in the next. The 4724 flies caught were classified in 17 different species, in the field laboratory, just after the collections. No *scute* male of *D. willistoni* was observed among the 344 individuals of this species collected (154 males).

The significance of this fact appears if we consider that in March 10 and 11, 1956, in a total of 5455 flies collected at wood I, 1362 were of *D. willistoni* wild phenotype, plus 238 males and 57 females *scute*. The frequency of the *scute* gene was, thus, still very high : $q=0{\cdot}2088$ for males and $q=0{\cdot}3039$ for females. A few days after (March 23) wood II was visited and among 879 flies, collected with great difficulty due to the low temperature prevailing, 18 were *D. willistoni*, wild type.

The 1957 samples of wood I contained 2380 *D. willistoni* and no *scute* individual was observed. This was noted on the first collection of this year ; thus, no attempt was made to study the possible occurrence of this gene in wood II.

Another source of evidence about *D. willistoni* isolation in these woods was obtained by simple collecting experiments. In four different instances, 5 to 8 banana traps have been placed outside them, in a

straight line from the edges toward the grassland (woods I and III). The occurrence of flies there was limited to the mornings (until 10 a.m.) and *D. willistoni* was found in the first trap only.

The isolation is particularly strong during the winter season, despite the winds, because *D. willistoni* do not fly when the temperature is around 13° C or lower. Consequently, the migration effect is negligible in the results we are going to describe now.

3. CHROMOSOMAL POLYMORPHISM OF TAINHAS NATURAL POPULATIONS

The absence, or rare occurrence, of inversions in the X chromosomes, and the relatively low average number of inversions per individual, characterises Tainhas and all other localities from Rio

TABLE 3

Significant differences of autosomal inversion frequencies between control and interracially hybridised natural populations of Drosophila willistoni

Chromosomes and inversions	Control and wood I, sample in 1956		Control and wood I, two samples in 1957		Control and wood IV, sample in 1956		Control and wood V, two samples in 1957	
	χ^2	P(1 d.f.)	χ^2	P(2 d.f.)	χ^2	P(1 d.f.)	χ^2	P(2 d.f.)
IIL-A . . .	5·17	<0·05	6·78	<0·05	—	—	7·06	<0·05
IIL-B . . .	4·55	<0·05	6·34	<0·05	—	—	7·38	<0·05
IIL-C . . .	—	—	14·99	<0·001	—	—	—	—
IIL-D . . .	—	—	—	—	—	—	6·05	<0·05
IIL-H . . .	—	—	—	—	11·47	<0·01	—	—
IIR-E . . .	15·84	<0·001	—	—	—	—	—	—
III-B . . .	—	—	8·68	<0·02	—	—	6·26	<0·05
III-C . . .	11·54	<0·001	—	—	—	—	—	—
III-F . . .	12·49	<0·001	—	—	—	—	—	—

Grande do Sul to Argentina so far studied (Dobzhansky, 1957 and Cordeiro, 1959).

In the 1956 control wood III sample some inversions occurred in the X chromosomes (XL-A, XR-C and XR-D) ; but they did not reappear in 1957 (table 1). This fact is not significant however, due to the low frequency of these inversions in Tainhas. The same is true for several autosomal inversions, III-I_1 appearing in 1956 and not in 1957 and, inversely, III-B_1, H, M, and N occurring in 1957 and not in 1956. Nevertheless, the absence in 1956, followed by a relatively high frequency of inversions IIR-A and IIR-C in 1957 is probably significant. In the total of 26 different inversions recorded for either sample, only three showed significant χ^2 divergency. All three decreased in frequency (tables 1 and 2).

Drosophila willistoni relative frequency increased from $3{\cdot}49 \pm 0{\cdot}36$ per cent. in 1956 to $64{\cdot}76 \pm 1{\cdot}80$ per cent. in 1957, in a total of 2519 and 698 flies collected respectively. This increase is not apparently correlated with the observed changes in inversion frequencies.

The composition of the inversions in Tainhas are in agreement, in a general way, with the data obtained by da Cunha, Burla and Dobzhansky (1950) and da Cunha and Dobzhansky (1954) for four localities of Rio Grande do Sul.

4. THE COMPOSITION OF THE F_1 HYBRID NORTH/SOUTH STOCK RELEASED

The stock released in wood I was heterozygous for two " new inversions " (XL-D and XR-E) as far as the populations of Tainhas are concerned (table 1), and the X chromosomes of females carried in single dose the visible mutant *scute*. The majority of the hybrids N/S proved to be heterozygous for these two new inversions, and carried high frequencies of the autosomal inversions IIL-A, IIR-E, III-C, III-F and III-J. The average number of inversions per female, $7{\cdot}50 \pm 0{\cdot}31$, were more than double in comparison with the control populations. Two other releases were made, in woods IV and V. The composition of this stock included a visible autosomal mutant gene *ebony* in heterozygous condition and high frequencies (see table 1) of the " new " inversions XL-B_1, XL-D, XL-F and XL-G. The inversions XR-C and XR-D, also present in high numbers, had already been found in the control population in 1956, though with lower frequencies. No increase in the inversion content of the left arm of the second chromosome was observed in relation to the Tainhas natural populations, but the stock released carried as much as 65·6 per cent. of IIR-E, a common inversion in the northern races but relatively less frequent in southern populations (Dobzhansky, 1957). The III chromosome presented a great increment of the A and J inversions. The mean number of inversions per individual female was even higher than in the *scute* bearing stock, i.e., $8{\cdot}36 \pm 0{\cdot}41$.

5. THE SHIFT FROM BALANCE PRODUCED IN THE TAINHAS NATURAL POPULATION

The N/S stock released at the isolated wood I and wood IV, produced a temporary shift from balance, changing significantly the inversion frequencies during 1956. The results obtained can be seen in tables 1 and 3. The first sample in wood I was made 23 days (about 2 generations) after the release of the hybrid N/S flies. The frequency of *D. willistoni* in the total of flies collected then (5160 individuals) was $30{\cdot}4 \pm 0{\cdot}61$ per cent., as compared to $4{\cdot}3 \pm 1{\cdot}7$ per cent. found before the release. This increase occurred also in the control wood.

The " new " X chromosome inversions introduced in wood I were XL-D and XR-E. They appeared in fairly high frequencies in 1956, decreasing to low levels in 1957 (table 1) ; however they were not lost after the 1956 winter. Inversion XL-F did not appear in the stock released, or in the 1956 sample, but was present in the 1957 second

sample at the low frequency of 2·2 per cent. This inversion probably was present at a low frequency in the released stock and in the other samples. The " new " inversions released in woods IV and V (table 1) appeared only in the first 1956 sample. The second 1956 sample was too small to be significant, but the two 1957 samples, with 102 individuals analysed, confirmed the absence or extreme rarity of these inversions.

Several other inversions, present at very high frequencies in the hybrid North/South F_1, and occurring at Tainhas in lower frequencies, showed an increase, followed by a decrease and return to the control wood III level. Some cases should be emphasised. The comparison between the control wood III samples of 1956 and 1957, mentioned before, disclosed a significant decrease of IIL-D, IIL-H, and III-B (table 2). On the other hand, the comparison between the controls with the experimental woods' samples showed a very different picture. In wood I the significant decrease of IIL-A (1956-1957) was opposed by an increase in frequency of IIL-B (1956-1957); IIL-C (1957); IIR-E (1956) and III-C (1956). Inversion III-F increased in frequency in 1956 and declined in 1957; and finally III-B was present in high frequency in the first sample of 1957 and dropped down in the 2nd sample (see table 3).

Woods IV and V presented a similar picture. The only significant contrast in wood IV, however, is the 1956 decrease in frequency of IIL-H compared with the control wood III in 1956 (table 3). This inversion was not detected in the first sample of wood V in 1957 and was found in 5·9 per cent. of the second sample (table 1). This frequency is significantly lower than that found in wood IV in 1956. IIR-E, which is common in northern populations, had its frequency raised after the release in wood IV in 1956, but was reduced to control values in wood V in 1957. The same can be said of inversions III-C and III-J (table 1).

There are significant χ^2 differences among the inversion frequencies of the three after the release samples of wood I, contrasting with more uniformity in woods IV and V. Thus, in wood I, after the initial rise in frequency, there were significant decreases in the occurrence of inversions XL-D, XR-E, IIL-B, IIL-H, IIR-E, and III-C. In woods IV and V, IIR-E and III-C presented also a significant decrease, while the two samples of wood V were significantly different in regard to the frequency of IIL-B.

Several differences were noted between the 1957 wood V samples and the control of the same year (table 3); but because no sample of this wood was collected in 1956, and some significant differences were found also between the controls, no clear conclusion can be drawn from them.

The first samples after the release of the hybrids in woods I and IV showed a significant increase in the mean number of heterozygous inversions per individual female (table 1). However, a recurrence to

control levels was observed in 1957. The number of males studied was too small to disclose significant differences.

6. DISCUSSION

The comparisons made by Dobzhansky and Wright (1947) shows that *D. pseudoobscura* has the same dispersion rate as *D. funebris*, studied by Dubinin and Tiniakov (1946). On the other hand, *D. willistoni* has a significantly lower dispersion rate, when compared with *D. pseudoobscura*, according to Burla *et al.* (1950). This is important to consider in connection with the results reported here. The isolation among the woods' populations of Tainhas is an important condition, satisfied in our experiments.

Dubinin and Tiniakov (1946) reported an experiment in some points similar to ours. They gave some indirect evidences about the isolation of the Kropotovo population of *D. funebris*, which received 100,000 individuals homozygous for the II-1 inversion. The frequency of the individuals homozygous for this inversion increased one month after the release and decreased rapidly from July to October. The heterozygote increment occurred at the expense of both homozygous II-1 inversion and standard. The last arrangement was predominant before the release. Natural selection favoured the heterozygotes, according to Dubinin and Tiniakov (1946). Why it had not favoured them before, they did not try to explain. The trend toward the elimination of the homozygotes II-1 was very strong, and it is not clear if stabilisation of the inversion frequencies occurred at the end of the experiment. This may suggest that the final results consisted in a return to the former composition of the original population.

Five of the seven northern population inversions introduced in three semi-isolated southern natural populations of *Drosophila willistoni* have been lost, one year after the release. XR-E, lost in woods IV and V, remained in wood I. The fact that the majority of the inversions did not survive in the populations conforms with the concept that they have adaptive functions. The frequencies of *D. willistoni* inversions are correlated with ecologic opportunities, according to da Cunha, Burla and Dobzhansky (1950), and da Cunha and Dobzhansky (1954). XL-D, retained at Tainhas, was one of the heterotic sex-linked inversions studied by da Cunha (1955) in population cages. This inversion has been observed by da Cunha and Dobzhansky (1954) in Melo, Uruguay, although it has not been detected at the Tainhas control populations.

The rejection of northern gene arrangements suggests that inversions cannot be used as markers to determine the probable centre of origin of the species, as proposed by da Cunha, Burla and Dobzhansky (1950). Vavilov (1926) correctly based his hypothesis about centres of origins on the relative amount of *adaptively neutral* traits, which accumulates with time.

Turning now to inversions IIR-E and III-J, studied by Dobzhansky and Pavlovsky (1958) in experimental populations of *Drosophila willistoni* from the Brazilian localities of Recife and Guaramiranga, we may recall that in our experiment the release in 1956 raised the IIR-E frequencies to about 20 and 30 per cent., respectively, from the control value, these frequencies returning to normal levels again in 1957. The occurrence of III-J was not affected by the release. The results of Dobzhansky and Pavlovsky (1958), on the other hand, showed a clear significant loss of heterozygosity of the reciprocal hybrid populations Guaramiranga/Recife and Recife/Guaramiranga. The interpretation we propose for these apparently conflicting results is the following: the racial hybridisation produced, in both experimental and natural populations, a breakdown of coadapted gene complexes. However, due to the degree of environmental diversity, the natural population of Tainhas retained its characteristic amount of polymorphism. Contrasting with this, the stabilising selection active on experimental cage populations reduced the racial mixture to a more homogeneous population, more likely to be fit in the more constant and simpler cage conditions (Thoday, 1959 ; Lewontin, 1958).

The stability observed by Dobzhansky and Pavlovsky (1958) in the experimental cages with the " pure " racial stock of Recife or Guaramiranga, on the other hand, suggests that a balanced population tends to maintain its structure even when transferred from its original heterogeneous environment to a more homogeneous one. Many experimental attempts to remove populations rapidly from " adapted average values for specific traits, either by artificial selection or by changes in the breeding system, are counteracted by natural selection, which is directed towards the maintenance of a phenotypic balance between fitness determining characters " (Lerner, 1954). The Tainhas experiment may be described as an attempt to increase heterozygosity; this has been counteracted by natural selection, also.

7. SUMMARY

1. An experimental introduction of about 60,000 F_1 hybrids of North/South races of *Drosophila willistoni* was performed in three isolated small woods at the grassland region of Tainhas, Rio Grande do Sul, Brazil.

2. Cytologic analysis of polytenic salivary gland chromosomes of the F_1 North/South hybrids showed a very high frequency of six " new " inversions in the X chromosome, not present at the Tainhas region (XL-B_1, XL-D, XL-F, XL-G, XL-H and XR-E), and more than 50 per cent. of inversions XR-C, XR-D, IIL-A, IIR-E, III-A and III-J.

3. The first sample after the release of the N/S F_1 hybrids, showed a relatively high frequency of the " new " X chromosome inversions introduced and a significant increase of some autosomal inversions.

4. The samples of the following year disclosed an opposite situation, since most of the introduced genetic novelties have been lost or decreased at too low frequencies to be detected. The only surviving "new" inversions were in one of the three woods studied (wood I, inversions XL-D and XR-E).

5. The autosomal inversions that increased in frequency after the release of the hybrids, dropped down to the control level of frequencies one year after.

6. The average number of heterozygous inversions per individual presented a similar increase and return to normal values.

7. Several conclusions can be drawn from this experiment. *First* we may say that a shift from balance was produced in the frequency of heterozygous inversions. *Second*, that the genetic structure of outbreeding natural populations has strong homeostatic properties, that tend to reject abrupt or drastic changes even if these are for a further increase in heterozygosity. *Third*, that the differences observed between natural and experimental interracial hybridisation experiments showed the importance of environmental variability in the origin of genetic polymorphism.

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CHROMOSOME STRUCTURE IN *PERIPLANETA AMERICANA*

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In general paraffin methods are inferior to squash techniques for chromosome preparations. From a study of preparations made with these older paraffin methods Sharma, Parshad and Sehgal (1956, 1959) have criticised our conclusions regarding the organisation of the chromosomes in the American cockroach *Periplaneta americana*. In this paper we intend summarising the evidence which indicates that many of their observations are incorrect. In consequence we consider their arguments invalid.

Sharma *et al.* make the following claims :—

1. During the anaphase of mitosis and meiosis chromosome ends lead the way to the poles and all the centromeres are terminal.
2. The prophase of meiosis is cut short by the early dissolution of the nuclear membrane and the precocious activity of the division centres. As a consequence, chromosome replication occurs at pro-metaphase. On their view this means that there is insufficient time for chiasma formation and consequently diplotene-diakinesis is not represented in the meiotic sequence. They also conclude that homologues are held together, in the absence of chiasmata, " by the force of attraction due to the singleness of each homologue ".
3. Colchicine treatment induces diplotene-diakinesis stages (John and Lewis, 1957) with chiasmata because it prolongs prophase.

Our own observations and conclusions are described below.

1. CENTROMERE POSITION AND BEHAVIOUR

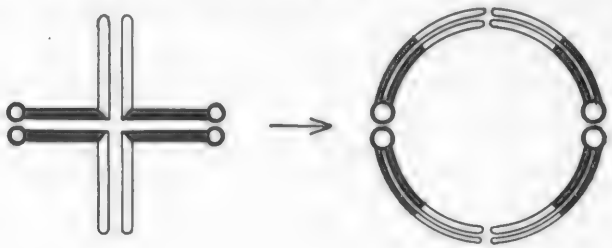
(i) *Chromosome morphology*

Constrictions are visible in all the chromosomes at mitosis in untreated cells (plate, figs. 1 and 2) and they are particularly clear following colchicine treatment (plate, fig. 3). Many spindle inhibitors cause selective extension of chromosome constrictions (Sokolow, 1939 ; Tjio and Levan, 1950).

One of the largest chromosomes in the haploid complement has two constrictions, the one median, the other submedian (plate, fig. 4). We have already suggested that this may be the nucleolar organising chromosome (John and Lewis, 1959). The constrictions of the other

chromosomes are median to subterminal in position. We consider these to be primary, centric, constrictions. On the view of Sharma *et al* that the centromeres are terminal, one would be forced to conclude that there are thirty-five secondary constrictions in the chromosome complement of male *P. americana*. This would surely be a record.

Interstitial constrictions are often visible in all the chromosomes at pachytene of meiosis and these also are extended by colchicine treatment (plate, figs. 7 and 8). Separation of the homologues begins at these constrictions and proceeds towards the chromosome ends (cf. figs. *a-d*, Lewis and John, 1957*a*). A similar behaviour is figured (Suomalainen, 1946, plate II, figs. 11 and 12) in untreated cells of *Periplaneta australasæ*. At prophase and pro-metaphase of the second meiotic division sister chromatids are also associated interstitially



TEXT-FIG. 1.—Origin and nature of the interchange ring of four possible in an individual with telocentric chromosomes : (i) zygotene, (ii) pro-metaphase. Interstitial segments shown solid.

although the crosses so formed are somewhat deformed by matrical stickiness (plate, fig. 5).

All the evidence from chromosome morphology is thus against the conclusion that the centromeres are terminal. This evidence in itself does not of course disprove terminal kinetic activity, but it does prove that if there is such activity it is a secondary and not a primary event. Neo-centric activity of chromosome ends has been described, though only at meiosis (Prakken and Müntzing, 1942); but in *P. americana* the evidence from chromosome behaviour is as unequivocal as that from chromosome morphology in showing that the centromeres are not at the ends (plate, fig. 6). The observations of Suomalainen (1946, fig. 61) are also in agreement with our findings.

(ii) *The behaviour of interchange rings*

The populations of *P. americana* which we have studied are characterised by having a high frequency of interchange heterozygotes (Lewis and John, 1957*b* ; John and Lewis, 1958).

Where centromeres are terminal an interchange multiple of four with terminally associated members would have the structure shown in text-fig. 1. This holds whether the maintenance of association is dependent on terminal chiasmata, terminal attraction independent of chiasma formation or both. This means that the multiple would be maintained by attraction between homologous centromeres even at metaphase. For Sharma *et al.* this poses no particular problem since on their view the formation of ring bivalents must depend on a similar attraction.

One of the factors affecting the fertility of interchange heterozygotes is the orientation of the multiples. A multiple must be arranged in a way such that adjacent centromeres move to opposite poles. The probability of disjunctional orientation is increased, especially for closed multiples, when the effective inter-centromeric distances are equal. In multiples with only terminal associations this condition is fulfilled when the chromosomes are equal in size and *iso-brachial*.

Multiples similar in many respects to the hypothetical example drawn in text-fig. 1 have been described in the short-horned grasshopper *Trimerotropis citrina* where the centromeres are very near the chromosome ends (Carothers, 1931). Theoretically, ring multiples could be formed in acrocentric types like this in two ways :—

(*a*) By the formation of chiasmata (or some other type of association) not only in both pairing segments but in both interstitial segments also. In this event centromeres would be absent from the closed loop. They would occur in half-loops which, because of their rotation, would lie at right angles to the closed loop. In this position homologous centromeres co-orient but the presence of an association in the interstitial segments permits little or no interaction between non-homologous centromeres, and the rigidity of the multiple means that adjacent non-homologous centromeres would often move to the same pole (see fig. 89, p. 264, Darlington, 1937). And this, in turn, means chromosome non-disjunction for those segments proximal to the interstitial chiasmata (and for all segments in the absence of chiasmata) together with chromatid non-disjunction for those segments distal to them. The multiples of this type described by Carothers (1931) invariably behaved in this manner.

(*b*) A closed multiple could also be formed in a type with nearly terminal centromeres by the formation of chiasmata (or some other kind of association) in the pairing segments and in both short arms. In this case the centromeres would lie in the same plane and hence interaction could possibly occur. Where the second arms are very short, as they are in *T. citrina*, a multiple of this type is not expected to occur often. Indeed it is difficult to ascertain from the early work of

Carothers whether such a multiple was found. We think it probable, however, that the multiple in her fig. 35f is of this type and it was found only once. Whatever its exact structure, its orientation was non-disjunctional, like all the multiples described by Carothers irrespective of their form. We would conclude that this was so because homologous centromeres were too close together to co-orientate. In fact a multiple of this type is mechanically equivalent to a ring bivalent.

That centromeres can be too close for efficient co-orientation is indicated by the work of Revell (1947) and Rees (1955). The former found that in spiders, where the congression of polarised chromosomes is not in concert, bivalents with distally localised chiasmata congress first. Similarly in rye, Rees found that bivalents with proximally localised chiasmata were often delayed in their congression (see also Lewis, 1958).

Where multiples are made up of chromosomes of unequal size centromeres are not equidistant and in many cases those which are close together move to the same pole even when this leads to numerical non-disjunction (*cf.* fig. 52, p. 152, Darlington, 1937).

It is true that the pre-metaphase stretch, which in our opinion facilitates disjunction, is much more pronounced in *P. americana* than in short-horned grasshoppers. But it is very unlikely that the stretch would have much effect where the centromeres are terminal.

All these considerations indicate that the evolution of a system of permanent interchange hybridity, in a type with terminal or even nearly terminal centromeres, is so improbable that the possibility can be neglected. We trust that our investigations of some three hundred individuals from eleven populations of *P. americana* provide sufficient evidence that such a system obtains in the wild and cultured populations of this species in Great Britain. In this connection the populations studied by Sharma *et al.* would provide an interesting comparison with our coal mine populations. Far from being confined, the train compartment populations can move much more rapidly and farther than their own locomotory powers permit. This presumably leads to greater outbreeding. It is interesting to note, therefore, that Sharma *et al.* do not seem to have observed interchange multiples, though this too may be a result of the obvious handicap imposed by their consistent use of microtomy.

2. CHROMOSOME REPLICATION

There is no general agreement as to the time of DNA synthesis during the meiotic cycle and it appears to occur at slightly different times in different organisms (Moses and Taylor, 1955 ; Taylor, 1959). Opinion is also divided with regard to the stage at which recombination occurs, but it is generally accepted that both replication and

recombination are completed before diplotene. Even at this stage, however, the four chromatids and their chiasmate associations can often be resolved only in the most favourable material. This means that replication must occur during or before pachytene.

Because of the contracted state of the late pachytene bivalents (*contra* Sharma *et al.*) and their lampbrush appearance during the initial pre-stretch phase, we have found it impossible to resolve the chromatids at these stages in *P. americana*. Therefore we would ask Sharma *et al.* what observational evidence they have for concluding that replication in *P. americana* does not occur until pro-metaphase (Sharma, Parshad and Sehgal, 1956). We find it difficult to believe that they have been able to resolve chromatids in preparations where the constrictions have remained undetected.

Their conclusion can then only be based on the evidence of the continued parallel pairing of homologues. Hughes-Schrader (1943) has, however, already shown that this situation obtains during the non-chiasmate meiosis of the male *Callimantis* after the chromosomes have replicated. Sharma *et al.* certainly comment on Hughes-Schrader's observation, but then proceed to ignore it by concluding that the " diplotene-diakinesis " suppression in *P. americana* can also be explained, like that in *Callimantis*, as due to the persistent singleness of each homologue throughout prophase. In fact they accept the suggestion made by White (1938) before Hughes-Schrader's (1943) observations were published. Even White himself no longer believes in this (White, 1954, p. 231).

There is no evidence to show or even suggest that chromosome replication in *P. americana* takes place at prometaphase and there is therefore no reason to entertain the suggestion made by Sharma *et al.* concerning the induction of diplotene-diakinesis by colchicine treatment. On the contrary, if the chromosomes are studied during the pre-metaphase stretch, especially in its early stages, it can be seen that the chromosomes are essentially similar in structure to those found in *c*-treated cells. They differ from them in only one respect, *viz.* their distortion, which is an inevitable consequence of the violent pre-metaphase movement. Colchicine treatment by inhibiting the spindle prevents this movement, but it does not produce a stage totally different from that found in untreated material. During the early stages of the pre-metaphase stretch the bivalents are lampbrush in appearance and the homologues are separated for the greater part of their length. The bivalents at induced diplotene are of the same basic form (*cf.* plate, figs. 2 and 6, John and Lewis, 1957).

3. BIVALENT ASSOCIATION

Our earlier studies on *P. americana* led us to conclude that chiasmata are formed at least on occasion, though they must be localised near the chromosome ends in the majority of cases. Moreover matrical stickiness

and terminal affinity may maintain chromosome association even in the absence of chiasmata.

The strength of the latter forms of association may be judged from the consistent formation of terminal adhesions at first ana-telophase (plate, figs. 6, 9 and 10). Sharma *et al.* use these bridges to support their contention of precocious chromosome despiralisation. But it is clear from fig. 6 of the plate that the body of the chromosome is not despiralised at all; the bridges are the consequence of the persistent association of the ends of the chromosomes only.

Because chiasmata are clearly evident in some other cockroach species Sharma *et al.* argue that there is no reason why they should not be equally obvious in *P. americana* also. But there is every reason why they should be difficult to detect. Those factors which make it impossible to resolve chromatids, seriously interfere with the detection of chiasmata too. An even greater impediment, however, is the matrical stickiness and the occurrence of pre-metaphase stretch immediately after pachytene. As we have already pointed out, the bivalents at this time are not basically different from those at induced diplotene-diakinesis. Particularly pertinent, therefore, is the fact that during the premetaphase stretch (plate, fig. 11), at metaphase (plate, fig. 9, Lewis and John, 1957*b*) and particularly at induced diplotene-diakineses (fig. *e*, Lewis and John, 1957*a*) subterminal associations can sometimes be seen.

In two other cockroach species where diplotene-diakinesis is a typical stage in the meiotic sequence, namely *Leucophæa maderæ* (Mathey, 1951) and *Blaberus discoidalis* (John and Lewis, 1959) chiasmata can be satisfactorily resolved. In the former they terminalise quickly and in the latter they are confined to terminal euchromatic segments.

For the reasons given earlier it is not permissible in the absence of supporting evidence, to conclude that chromosome replication is delayed in those cockroaches where diplotene-diakinesis is absent. The minimum conclusion is that the pre-metaphase stretch, where it occurs immediately after pachytene, is an impediment to the observation of chiasmata; there is nothing to show that it interferes with their formation. The problem of resolving distally localised, rapidly terminalising chiasmata is by no means confined to cockroaches (John, 1957).

The situation is clarified further by the work of Callan and Jacobs (1957) on *Mantis religiosa*. The males of this mantid have a diploid complement of $24+X_1\ X_2\ Y$ and, as in *P. americana*, there is a post-pachytene stretch. The homologues remain parallely paired until the stretch and chiasmata cannot be distinguished, except for occasional subterminal associations. There are, however, two lines of evidence which in particular indicate that chiasmata are formed:

(*a*) Pairs of heterobrachial chromosomes may be associated by only their short arms. A force operative in the absence of chiasmata would be expected to separate short arms before long ones.

Plate

FIG. 1.—Mitosis in a female blood cell (untreated, × 1600).

FIG. 2.—Male mitosis (untreated, × 1600).

FIG. 3.—Male c-mitosis (Colchicine 2 hr., × 1600).

FIG. 4.—Autosome with a secondary constriction (Colchicine 3 hr., × 3500).

FIG. 5.—Pro-metaphase II (untreated, × 1600).

FIG. 6.—Anaphase I (untreated, × 1600).

FIGS. 7 and 8.—C-pachytene in homozygous cells (Colchicine 4 hr., × 1000).

FIGS. 9. and 10.—Telophase I (untreated, × 1000).

FIG. 11.—Premetaphase stretch (R IV+14 II+X), note " chiasmate " bivalent (arrowed) and " lampbrush " nature of the autosomes (untreated, × 1000).

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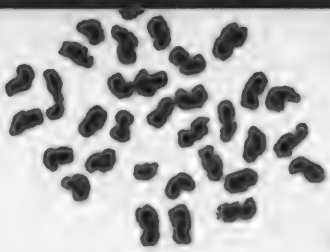

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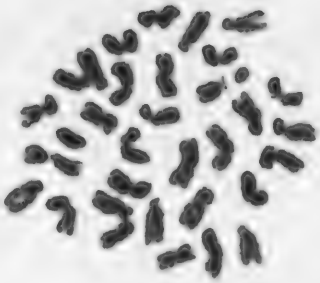

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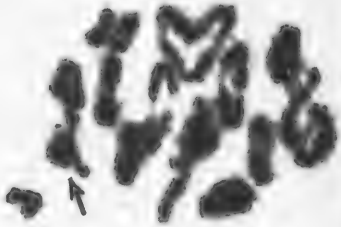

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(*b*) Zygotene pairing is regular and complete; nevertheless univalents occur at metaphase. Univalent sex chromosomes are three times as common as unpaired autosomes though there are six times as many autosomes in the complement. What is more, X_2 univalents are twice as common as X_1 univalents. Both these observations can be readily explained on the assumption that metaphase association is dependent on chiasma formation: they then merely require the assumption that the pairing segments of the sex chromosomes are shorter than those of the autosomes and that of X_2 is shorter than its counterpart in X_1.

In summary, the organisation of the chromosomes in *P. americana* is not, as Sharma *et al.* intimate, purely a matter of opinion: it is a matter of observation and inference. We do not " appear to believe " that the centromeres are interstitial: we are convinced by observation that they are, and these observations leave no room for ambiguity. We are equally confident that colchicine-induced diplotene-diakinesis has its distorted counterpart in the normal meiotic sequence. Finally, we do not assume that chiasmata have become completely concealed by a prolongation of parallel pairing. On the contrary, we have seen and figured what in other organisms would be regarded as chiasmata. But we suggest that their terminal localisation together with matrical stickiness and the occurrence of the post-pachytene stretch makes it difficult to detect them consistently.

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PHYSIOLOGICAL DIVERSITY AMONGST TRYPTOPHAN MUTANTS IN *NEUROSPORA CRASSA*

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1. INTRODUCTION

The discovery of complementation between alleles and especially the occurrence of patterns of relationship which can be reduced to linear complementation maps (Catcheside and Overton, 1958 ; Pateman and Fincham, 1958 ; Woodward, Partridge and Giles, 1958) has made it advisable to examine a wide range of genes for the phenomenon. Two purposes prompt such a search, one that the frequency of occurrence is of interest and the other that instances more favourable for genetical and biochemical analysis may be found. Mutants affecting the biosynthesis of tryptophan are of especial interest in this connection. The genetic control of tryptophan synthetase is becoming extremely well understood and, indeed, after we had already gathered a large number of new tryptophan mutants, Lacy and Bonner (1958) reported the occurrence of complementation between some of the *td* mutants. They find that complementation appears to be restricted to those mutants, each of which produces a protein (CRM) which is sufficiently similar to tryptophan synthetase to cross-react with the specific antiserum to it.

It should be mentioned that the name of tryptophan synthetase has been changed from tryptophan desmolase, by which it was first known and after which the controlling gene was given the symbol *td*. The gene has also been denoted *tryp*-3, as it was the third one discovered to affect tryptophan synthesis.

It has also become clear that indole glycerol phosphate is the compound from which tryptophan is formed, and that free indole is not itself an intermediate in synthesis (Yanofsky and Rachmeler, 1958). It was suggested by Yanofsky and Rachmeler (1958) that synthesis proceeds by the two coupled steps (1) indole glycerol phosphate+ tryptophan synthetase→indole tryptophan synthetase+triose phosphate. (2) indole tryptophan synthetase+L-serine→L-tryptophan +tryptophan synthetase.

If these constituent processes are capable of independent loss by mutation, a variety of physiologically different mutants would be

* Work begun in University of Birmingham while holder of a Dominion Travelling Fellowship of the Nuffield Foundation.

possible. Consequently, the discovery of *td* mutants able to utilise indole was not completely unexpected.

2. MATERIAL AND METHODS

A large number of mutants were obtained from the strain Emerson *a* (5297) by a modified technique of filtration enrichment (Catcheside, 1954). The modifications were (1) the use of a macroconidial strain, a fair proportion of whose conidia are uninucleate, (2) agitation of the suspension by bubbling filtered air through the liquid medium, (3) use of Vogel's minimal medium (1956) containing a normal amount of sucrose but no sorbose and (4) maintenance of the cultures in a water bath at 25° C. The filtered air was pumped through a Pasteur pipette inserted into the culture vessel (a boiling tube); to secure continued suspension of the conidia, and separately growing colonies, it was found necessary to have the orifice of the Pasteur pipette very close to the bottom of the tube. After the final filtration, suitable amounts of the suspension were plated in sorbose minimal medium, supplemented with 300 mg. tryptophan per litre. Colonies were isolated to individual tubes and tested on sorbose minimal medium to identify mutants. Tests of the ability of mutants to use anthranilic acid or indole were made on sorbose minimal medium supplemented with one of these compounds.

Heterocaryon tests were made by superimposing small drops of dense suspensions of conidia of the two strains to be tested with one another. When a large number of mutants had to be tested in all combinations, this was done on petri dishes containing sorbose minimal medium. Each dish was marked on the base with a grid of twenty-five squares, each half an inch square, and in five rows of five each. The individual dish and orientation of the square was identified by a code consisting of a letter and a number on adjacent sides. Small drops of conidial suspension were applied, a different strain to each horizontal row. When these drops had dried, they were overlaid with drops of other strains, a different one to each vertical row. In testing n different mutants, all the $\frac{1}{2}n(n-1)$ combinations, as well as the n control inoculations, would be set up within a relatively short time, using Pasteur pipettes with finely drawn nozzles to dispense the drops. In practice, not more than about fifty mutants can be managed conveniently in one such test. The plates were incubated at 25° C and examined at intervals for signs of growth. More critical tests were done singly on plates with control drops of the constituents.

3. RESULTS

A large number of mutants, well over 100, were isolated initially, but the number finally classified was only 77 (table 1). The remainder included some which reverted to wild type and many which grew very poorly and were not worth the trouble of outcrossing to recover better stocks. Much less work would be involved in obtaining further mutants *ab initio*. It is evident that mutations deleterious to growth or conidial production are very frequent, but there is no indication that they are correlated with particular kinds of biochemical mutations.

The tryptophan mutants were first classified into the three nutritional groups (a) those (tryptophan mutants) which could grow only if tryptophan was supplied, (b) those (indole mutants) which could grow on indole as well as tryptophan and (c) those (anthranilic mutants) which could grow on anthranilic acid as well as on indole and tryptophan. Next, each nutritional group was further subdivided, if it could be, by heterocaryon tests. Initially, one mutant was selected at random in each group and tested for its ability to complement each of the others

in the same nutritional group. All those with which it was negative would be judged as allelic with it. Those with which it was positive would then be further tested with one of their number in turn, and this process of division continued until all mutants in a nutritional group had been assigned.

These tests disclosed one group of tryptophan mutants, three groups of indole mutants and two groups of anthranilic mutants. Linkage tests and heterocaryon tests with standard tryptophan mutants showed that four of these six groups corresponded to previously known tryptophan mutants, but that two (one indole and one anthranilic) appeared to be new. Crosses were then made systematically to locate these presumed new genes.

The new indole group, represented by A45 and A78, was finally

TABLE 1

Schedule of tryptophan mutants of Neurospora crassa. *For full reference each number should be prefaced by a capital letter A.*

tryp-1		
	group A	A9, 48, 49, 51, 52, 56, 93, 94, 105, 107, 110, 121, 123
	group B	A20, 28, 38, 50, 62, 63, 117
	group C	A10, 65, 67, 82, 106
tryp-2		
		A4, 55, 60, 64, 80, 81, 95, 109, 112
tryp-3 (=*td*)		
	group A	A16, 17, 21-27, 29-34, 36, 41, 43, 44, 46, 59, 68, 75, 76, 79, 88, 89, 97, 100, 101, 104, 111, 120, 122
	group B	A72
	group C	A42, 113
	group D	A18, 69, 74, 102
	group E	A73
	group F	A45, 78
tryp-4		
		A5, 11, 35, 61

located in chromosome II at a position which suggested it was closely linked to *td* (=*tryp*-3). Each of these mutants were then tested for their ability to complement the 42 *td* mutants obtained in these experiments. It was found that each could complement 7 of the 42, but none of the others. It was evident, therefore, that by heterocaryon tests A45 and A78 are allelic to all the other *td* mutants.

In view of the discovery (Yanofsky and Stadler, 1958) that some *Escherichia coli* mutants, which have a modified tryptophan synthetase, marked by inability to utilise indole glycerol phosphate, can nevertheless utilise indole in place of tryptophan, it was obviously possible that A45 and A78 were of this type and one of them was sent to Dr. Yanofsky. It has been reported (Rachmeler and Yanofsky, 1959) that A78 forms a tryptophan synthetase which can convert indole to tryptophan, but is inactive in the reactions involving indole glycerol phosphate. Antibody neutralisation tests, performed with the tryptophan synthetase of A78, have shown that it is inhibited to the same extent as is the enzyme of the wild type.

The new anthranilic group, represented by A10, A65, A67, A82 and A106, was located in chromosome III at a position very close to *tryp*-1 (10575). Heterocaryon tests showed that they would complement some of our *tryp*-1 mutants but not most of them.

Thus it was found that both of the new groups of mutants were allelic to two of the four tryptophan genes already known. Each of the new groups represents a new physiologically distinct type at its respective locus. These loci will now be considered in more detail.

(1) *Tryptophan*-3

This is better known as the tryptophan desmolase (*td*) or tryptophan synthetase locus. Our collection contains 44 mutants at this locus. When tested in all possible pairwise combinations (946) it was found that some pairs formed heterocaryons which would grow on minimal medium. Altogether 19 pairs, involving 10 different mutants, showed complementation. The patterns of complementation between these

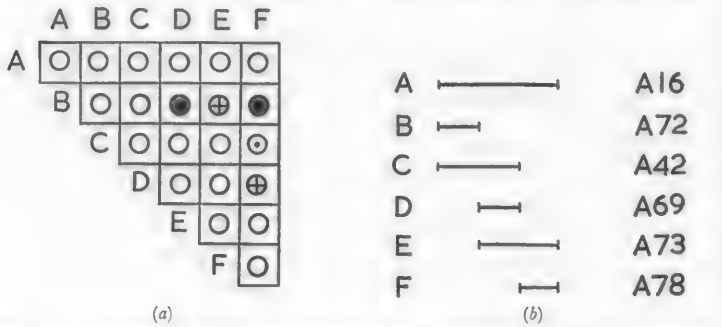

FIG. 1.—Complementation between different groups of *tryp*-3 mutants. (*a*) Observed complementations: solid circle=strong, inscribed cross=moderate, inscribed dot=very weak, open circle=no complementation. (*b*) Complementation map, with typical mutants.

ten mutants fall into five types. Including the large class of mutants, 34 in number, showing no complementation with any other allelic mutant, there are six patterns of reaction (fig. 1*a*). This neglects the more subtle differences of vigour, which will be discussed later. The two-dimensional diagram can be represented as a one-dimensional complementation map (fig. 1*b*). The principle used in its construction is that each class of mutant is represented as inactivating a section of a linear structure so that noncomplementing ones overlap and complementing mutants do not overlap. The map drawn in figure 1*b* is the only one possible that will represent the data of figure 1*a* on these rules.

It is interesting to notice that class F, made up of the mutants which can grow on indole, comes at one end of the complementation map. It remains to be shown whether any of the mutants in classes B, C and D, which are complemented by class F mutants, can carry out the

reaction, indole glycerol phosphate→indole + triose phosphate. Nevertheless, it seems clear that there is some degree of regional specialisation within the *tryp*-3$^+$ gene in respect of the two parts of the reaction converting indole glycerol phosphate + L-serine to L-tryptophan.

Amongst the nineteen heterocaryons that the ten complementing mutants can form, there is a considerable diversity in vigour. This is shown in two ways, one the speed of formation of a heterocaryon by superimposed drops of conidia and the other the growth rate or growth yield in a given time of an established heterocaryon on minimal

TABLE 2

Dry-weight yields of tryp-3 *heterocaryons, grown in submerged liquid cultures, expressed as percentages of the yield of wild type ; tr = trace of growth, unweighed, probably about* 0·1 *per cent. of the weight of wild type.*

	72	42	113	18	69	74	102	73	45	78
72	0	0	0	24·5	27·3	27·3	50·2	tr	33·8	26·4
42	0	0	0	0	0	0	0	0	0·2	tr
113	0	0	0	0	0	0	0	0	21·0	tr
18	0	0	0	0	0	0	0	0	0·1	1·3
69	0	0	0	0	0	0	0	0	1·4	tr
74	0	0	0	0	0	0	0	0	0·3	0·3
102	0	0	0	0	0	0	0	0	0·4	tr
73	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0

medium. The data shown in fig. 1 are intended to represent the vigour as assessed by speed of formation of a heterocaryon and its density of growth on solid medium. The growth yields have been determined in shaken conical flasks of minimal medium and expressed, in table 2, as percentages of the yield of wild type grown in the same way at the same time. The relative density of growth in submerged liquid culture does not always parallel growth on solid medium. Variation in vigour is shown not only between heterocaryons of different class relationship, but also between heterocaryons of the same class relationship. Thus of the eight heterocaryons possible between mutants of the D and F classes, A18 + A78 and A69 + A45 show some growth but

the remaining six are very feeble. Amongst the heterocaryons possible between A72 and the four mutants of class D, that involving A102 is about twice as vigorous as the other three. Thus the vigour of the heterocaryons, formed with group B and group F mutants, is a character which separates the four mutants of group D into subgroups.

(II) *Tryptophan*-1

When it was discovered that the new group of anthranilic acid mutants, five in number, were allelic to *tryp*-1, we had 20 mutants already assigned to this locus. Heterocaryon tests showed that the anthranilic mutants (class C of fig. 2*a*) would complement seven of these (class B of fig. 2*a*). The heterocaryons were formed quite quickly ; growth was often detectable at the end of one day and was well established in two days. The heterocaryons grew vigorously. They attained 83-91 per

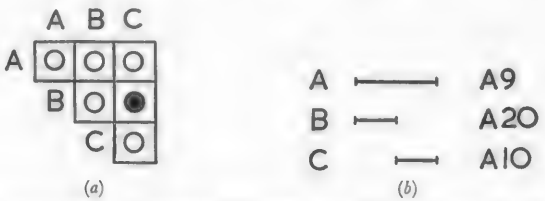

FIG. 2.—Complementation between different groups of *tryp*-1 mutants. (*a*) Observed reactions. (*b*) Complementation map, with typical mutants.

cent. of the dry-weight yield of wild type when grown in liquid minimal medium, on a shaker.

When tryptophan mutants are grown on slopes or in liquid medium in the presence of a limiting amount of tryptophan, the accumulation in the medium of substances that fluoresce in ultra-violet light is very marked. Mutants able to grow on tryptophan alone or both tryptophan and indole show a bright blue fluorescence. Mutants able to grow on anthranilic acid as well show a pale blue fluorescence.

If drops of culture filtrate are chromatographed, several substances may be distinguished, though most cannot yet be identified (table 3). The same compounds are accumulated by A20 (*tryp*-1 of the B class) as by A5 (*tryp*-4) ; these include anthranilic acid, the only compound identified positively. Mutant 10575 is probably also a member of class B. The mutant A10 (*tryp*-1 of the C class) and the mutants A9, A48, A49, A51, A52 and A56 (*tryp*-1 of the A class) resemble A4 (*tryp*-2) in the detectable compounds they accumulate.

Thus the new type of *tryptophan*-1 mutant (class C) differs from those previously known in three respects, (a) it can grow on anthranilic acid, (b) it can complement some, but not all, *tryp*-1 mutants and (c) it does not accumulate anthranilic acid and the other compounds characteristic of *tryp*-1, but resembles *tryp*-2 in this respect.

TABLE 3

Chromatography of culture filtrates of tryptophan mutants grown on Vogel's medium supplemented with 4 mg. tryptophan per 100 ml. Developed by descending chromatography on Whatman no. 1 paper, using 0·2 N ammonia-propanol (1 : 3) at 20° C.

Compound	R_F	Fluorescence in ultra-violet	Colour with Ehrlich's reagent	Accumulations					
				A5 (*tryp*-4)	A20, 10575 (*tryp*-1B)	A9, 48, 49, 51, 52, 56 (*tryp*-1A)	A10 (*tryp*-1C)	A4 (*tryp*-2)	Emerson *a* (wild type)
A	0·11	light blue*	—	+	+	o	o	o	o
B	0·14	indigo†	—	o	o	+	+	+	o
C	0·21	medium blue*	—	+sl.	+	o	o	o	o
D	0·35	light blue*	yellow	+	+	o	o	o	o
E‡	0·53	bright blue*	yellow	+	+	o	o	o	o
F	0·64	apple green*	—	+	+	o	o	o	o
G	0·65	blue†	—	o	o	+	+	+	o
H	0·67	blue†	—	+	+	o	o	o	o

Notes: * Towers ultra violet lamp, operating at 3650Å.
† Hanovia "Chromatolite" lamp, operating at 2537Å.
‡ Compound E is anthranilic acid.

4. DISCUSSION

The present study has shown that two of the four genetic loci concerned specifically with the control of tryptophan synthesis are physiologically complex. We cannot say that the other two are not, because only a few mutants have been examined in each case, namely nine *tryp*-2 and four *tryp*-4.

We do not propose to discuss the general problem of the mechanisms of complementation, the outlines of which have been considered elsewhere (Catcheside and Overton, 1958). However, there is one aspect of the relation to function that is particularly appropriate to the present material. Roman (1958) and Yanofsky and Stadler (1958) have suggested that the complexities, of the kind shown by complementation or by two related enzymic activities determined by the same gene, could be understood if two different products were controlled by overlapping segments of the chromosome (fig. 3). Mutations in the

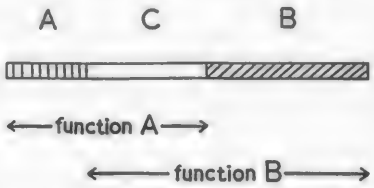

FIG. 3.—Diagram to show hypothesis of determination of two functions by overlapping segments of a chromosome.

common segment would be expected to eliminate both of the functions simultaneously, while mutations in either of the unique segments would be expected to eliminate only the corresponding function.

In the case of the *tryp*-3 mutants, complete explanation along these lines is impossible because the gene behaves as though there were three distinct functions, any one or all of which can be eliminated by a single mutation. It is not possible to have a system of overlapping segments, involving three functions, such that each has a unique part of the linear genetic material and a segment common to all three. It seems more likely that the function is a unitary, if complex, one in which different genetic defects may cause more or less extensive physiological defects. Part of the function may be left, as in A78, or none, as in its allele A16.

Most of the *td* mutants accumulate indole glycerol rather than indole (Lacy and Bonner, 1958) despite that several of them produce the protein CRM which cross-reacts with the antibody to tryptophan synthetase. Only td^2 and td^{71} accumulate indole; they belong to the same complementation class (Lacy, private communication). It may be that the altered enzymes, controlled by other mutants which produce CRM, while able to catalyse the reaction indole glycerol phosphate→indole+triose phosphate, leave the indole so tightly bound

to the enzyme that the reaction is stopped. Perhaps td^2 and td^{71} enzymes have a lowered affinity for indole and this results both in the accumulation of indole and in the prevention of the condensation of indole with L-serine to form tryptophan. It has recently been shown (De Moss and Bonner, 1959) that the partially defective enzyme produced by td^{71} requires pyridoxal phosphate to carry out the reaction indole glycerol phosphate + enzyme → indole-enzyme + triose phosphate, whereas normal tryptophan synthetase does not.

The genetic evidence suggests that *tryp*-1 parallels *tryp*-3 (=*td*) in its physiological complexity. In the latter, indole is not a free intermediate in the biosynthesis of tryptophan. The substrate of the reaction is indole glycerol phosphate and indole is a bound intermediate in the reaction as a whole. Certain mutants, such as A78, are able to bind and use indole, but either cannot bind indole glycerol phosphate or else cleave it, because they have an altered tryptophan synthetase. The fact that A10, and some other *tryp*-1 mutants, can bind and use anthranilic acid, while other alleles (group A) neither use nor accumulate anthranilic acid, suggests that this substance is not a free intermediate in the normal reaction controlled by the gene *tryp*-1^+. This reaction has been shown to involve a condensation between anthranilic acid and 5-phosphoribosyl-1-pyrophosphate (PRPP) to form a ribotide of anthranilic acid (Yanofsky, 1956). It now appears that this is only a part of the reaction catalysed by the enzyme controlled by *tryp*-1^+. It seems necessary to look for some other compound, perhaps a phosphate of anthranilic acid or a similar substance, which would be bound by this enzyme and cleaved to yield an enzyme-anthranilic acid complex which could then react with PRPP.

This evidence about the action of *tryp*-1^+ also indicates the relative sequence of action of *tryp*-1^+ and *tryp*-4^+. Since *tryp*-1^+ is concerned with the binding of anthranilic acid and *tryp*-4 mutants accumulate but cannot utilise anthranilic acid, the synthetic step controlled by *tryp*-4^+ must come after that controlled by *tryp*-1^+. *Tryp*-4^+ must be concerned with the binding of the ribotide of anthranilic acid, its rearrangement, the decarboxylation of the benzene ring and ring closure of the side chain to give indole glycerol phosphate. This appears to be a complex process and it would not be surprising to find that *tryp*-4 is also a complex locus.

5. SUMMARY

A set of 77 new tryptophan mutants, belonging to four genetic loci, have been classified physiologically. Complementation between alleles occurs amongst *tryp*-1 and *tryp*-3 mutants. The complementation map of the *tryp*-3 (tryptophan desmolase) mutants is complex; two of the mutants are of a new type able to grow with indole as well as tryptophan. The complementation map of *tryp*-1 is simple, but five of the mutants can grow with anthranilic acid. These five mutants do not accumulate anthranilic acid and other unidentified compounds,

which are accumulated by complementing *tryp*-1 alleles unable to utilise anthranilic acid. Non-complementing *tryp*-1 alleles neither accumulate nor use anthranilic acid.

Acknowledgments.—We are indebted to Mrs Anne Hollis and Miss Angela Neale for technical assistance and to Miss Elisabeth Percy, who drew the diagrams.

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A COMPARISON OF SELECTIVE METHODS AND A TEST OF THE PRE-ADAPTATION HYPOTHESIS

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1. INTRODUCTION

The capacity of some species to adapt to poisons introduced into their environment has been known for many years. The work is summarised in the review articles of Crow (1957), Milani (1957), and Brown (1958).

Earlier work with DDT has produced conflicting results on the hereditary basis, and manner of selection, of resistance (Crow, 1957; Milani, 1957; Tsukamoto, 1958). Different species of insects seemed to resist the poison by different genetic mechanisms. The same species, viz., *Drosophila melanogaster*, adapted by quite different hereditary changes in different laboratories under varying conditions of culture and selection. Some resistant strains proved to have the major portion of the resistance controlled by a single dominant locus or region (Ogaki and Tsukamoto, 1957). Other strains (Crow, 1957) have been shown to have their resistance based on many factors which exert their effects additively. Some of the strains were selected by exposure of adults to aerosols, some to DDT on paper, others by exposure of larvæ to DDT in the food medium, and some by topical application. With this variety of selective methods it is not surprising that a variety of hereditary responses resulted. Furthermore, Levene, Pavlovsky and Dobzhansky (1958) have shown that replicate samples of the same population may react differently under apparently identical conditions.

Insecticide resistance is genetically similar to drug resistance in bacteria, and shares in the continuing controversy over pre-adaptation (the presence in populations of individuals with some degree of hereditary resistance before exposure to the toxic agent) and post-adaptation (the development of resistance by all, or part, of a population by physiological adaptation, which may or may not be inherited, as a result of exposure to the toxic agent). The pre-adaptational view has been well substantiated, for bacteria, by the work of Cavalli-Sforza and Lederberg (1956), the clearest evidence arising from the production of resistance without exposure to the toxic agent by means of sib-selection. The best previous evidence in favour of pre-adaptation in Drosophila has been based on the inability of selection of any kind to

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produce significant resistance in highly inbred strains (Merrell and Underhill, 1956 ; Crow, 1957).

The present study was planned to throw light on three main points;

1. If the adaptation is, or can be, a purely selective process, based on hereditary variability already in the population, then it should be possible to select for or against the adaptation without ever exposing the organisms in the direct ancestral line to the selective agent.
2. Will different methods of selection applied to samples of the same starting population give variant results, even with similar culture conditions?
3. The various resistant strains of *D. melanogaster* produced in different laboratories had been selected and tested by different means. Some of these strains were obtained and cultured and tested along with the strains selected in the Madison laboratory. They can now be compared as to degree of resistance under the conditions of the Madison laboratory.

2. MATERIALS AND METHODS

The flies were tested for DDT tolerance by being exposed to DDT deposited on paper lining the vials in which they were confined. Papers were prepared with the following concentrations of DDT, in micrograms per square centimetre (10^{-6} μgm./cm.^{2}) : 1/25 (0·04) ; 1/5 (0·20) ; 1 ; 5 ; 25 ; 125 ; 625. Some were produced with 6050 for use in the population cages with direct selection.

The DDT-lined vials were assembled into " test sets." In the initial testing, of generations 0 and 1, a " test set " consisted of 5 vials with the concentrations : 1/25, 1/5, 1, 5, 25. The number of vials was later reduced to three, and two different kinds of " test set " were produced, the " high sets " with the concentrations : 1/5 ; 5 ; and 125 (later 1, 25, 625), and the " low sets " with concentrations : 1/25 ; 1 ; 25.

The lines selected for resistance to DDT were tested by exposure to the " high sets," those selected for susceptibility to DDT were tested by the " low sets." (Exposure 18 hours, counts 24 hours later.) By using test concentrations in regular multiples it was possible to apply " Karber's Method " (Cornfield and Mantel, 1950) to obtain an estimate of the dose which would kill half of the population (LD_{50}) represented by the flies tested in each set.

The LD_{50} values for each test set were treated as units of data in the statistical analysis. In preparing a test from three to six flies were required for each vial. Thus the LD_{50} value from a particular " test set " was based on the performance of 9 to 18 flies.

All testing was done on female flies, which give more reproducible results than males (Crow, 1954). In the case of the sib-selected lines it was not practical to use females of selected size, age, and condition because of the very small number of flies available in each culture and the lack of synchrony between cultures. This choice of material undoubtedly increased the variability of the results, but seems realistic in that it involved all adult stages rather than one selected age. All tests of DDT tolerance were made in incubators adjusted to 25° C.

The test sets were serially numbered and used repeatedly in rotation for several months. Thus each set was used the same number of times and changes in potency were presumed to be proportional to the various sets. Differences between sets were presumed to be equally distributed among all tested strains.

(i) *Methods of selection*

All selections were made on samples of a heterogeneous stock, produced from 17 genetically distinct laboratory strains and 9 wild type strains derived from 9 females collected at a local fruit warehouse.

Cage 1 had no exposure to DDT at any time during the course of the experiment. It therefore can be considered a control. Cage 2 had DDT added for lengthy periods, then removed for similar periods. After early intermittent application, similar to Cage 2, Cage 3 had continuous exposure to DDT. The DDT was introduced to the cages on papers identical with those used in the test vials (except that higher concentrations were used). The papers were left in the cages for one or more weeks, being replaced periodically.

(ii) *The sib-selection lines*

An initial group of 46 pairs of flies from the heterogeneous stock were placed in a similar number of food vials and allowed to produce progenies. Thus each pair of flies produced one progeny (=sibship=family) in one food vial. Each progeny was tested for DDT tolerance. After the tests were completed the progenies were ranked according to their tolerance, as evidenced by the group of females tested from each. The parents of the next generation were chosen from the untreated sibs of the tested progeny. The most resistant 5 progenies each provided 4 males and 4 females (mated serially) toward the establishment of the " High " line. The next 15 most resistant progenies each provided 2 males and 2 females (mated serially) to the " High " line. The 20 most susceptible progenies provided similarly for the establishment of the " Low " line. In each case the flies were pair mated, with one pair to each food vial. The result was 50 vials, each with a pair of parents, to produce 50 progenies to be tested in the next generation of each line. The process was repeated for each generation of selection, except that after generation 1 duplicate sets of parents were made up for each line, with the result that two " High " and two " Low " lines were available for selection. The High and Low lines were carried in pairs through all procedures, thus High Line 1 (HL1) and Low Line 1 (LL1) were tested at the same time and were raised on the same batches of food. Similarly High and Low Lines 2 (HL2 and LL2) were carried together.

About half-way through the selection a combination of events, most importantly a series of batches of poor food medium (the nature of the poorness is unknown), led to such a reduction of numbers in all sib-selection lines that mass mating was necessary to avoid losing the lines. This interval was named the " Mass Mating Hiatus," and is so designated on the appropriate figs. The minimum effective population size during this period is unknown, but is estimated to have exceeded 25 in all lines.

3. RESULTS

In all cases where selection was practised a change in degree of tolerance of DDT was produced. The amount of change, and the final degree of tolerance, varied considerably.

(i) *The population cages*

The data on the population cages are presented in fig. 1. Here the tests on the cages are summarised by two-month periods. The fortuitous variation in the values is apparent. Cage 2 seems to vary almost at random, but with a set of values quite different from those of Cage 1. These short-term changes almost certainly represent differences in the culturing of the samples taken from the cages, rather than actual changes of the cage populations. The critical point is the final difference

of tolerance between the cages. Cage 3 shows similar variation but a final greater tolerance to DDT than the other two.

If a period-by-period comparison is made between the three cages it will be noticed that the very high and very low points tend to occur together for all three. This is perhaps the best indication of a common external factor influencing the estimation of DDT tolerance of the three populations.

One may then conclude that in the case of these three cages continuous selection (Cage 3) was more effective than intermittent selection (Cage 2) as one might expect. In both cases an increase of DDT tolerance over that of the control (Cage 1) was achieved.

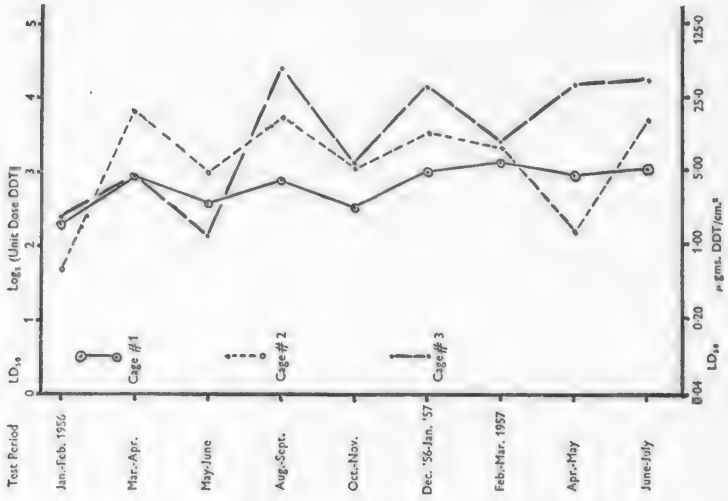

FIG. 1.—The comparative DDT tolerance of samples of 3 population cages, #1 unselected, #2 intermittent selection, #3 nearly continuous selection.

(ii) *The sib-selection lines*

The sib-selection lines were carried with selection for 14 or 15 generations, during the period of time that the population cages were being sampled. Fig. 2 shows clearly that the sib-selection was effective in HL1 and LL1. For the first three generations there was overlapping, and even reversal of relative positions of the values for the two lines. Then the lines quite clearly separated and remained so, with no hint of overlap at the 95 per cent. confidence level. Most of the difference between the two lines had been achieved by the 5th generation. Only relatively small gains were made later.

Fig. 3 presents a similar picture for HL2 and LL2. These lines were split off from HL1 and LL1 after generation 1. They demonstrate

quite clearly that similar selective procedures on genetically near-identical stocks can have very different results. The second lines appear to have achieved most of their separation at a time (both in strict chronology and in generations) when selection had become nearly ineffective in the first lines. The final degree of tolerance or susceptibility to DDT produced in the first and second lines was about the same.

During the selection period various mutant phenotypes appeared repeatedly, both in samples of the population cages and in the sib-selection lines. These were all recessives, some sexlinked, and had survived from the origin of the heterogeneous stock.

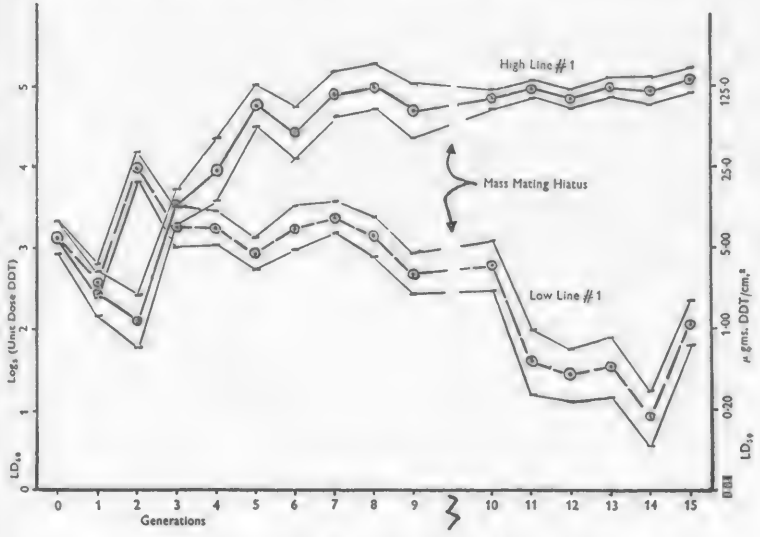
High Line #1
Mass Mating Hiatus
Low Line #1
Log10 (Unit Dose DDT)
LD50
μ gms. DDT/cm.²
LD50
Generations
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
0 1 2 3 4 5
0·20 1·00 5·00 25·0 125·0

FIG. 2.—The course of selection in High and Low Lines #1. Mean and 95 per cent. confidence limits of LD_{50}'s of Sibships in each generation of sib-selection.

At the end of the selection, stocks were made up from the sib-selection lines. For each Line two representative stocks, one extreme stock and a variable number of mutant phenotype stocks were prepared. The identity of the mutant phenotypes was not determined.

Fig. 4 presents a comparison of these various stocks derived from the sib-selected lines. It shows that the presence of the recessive mutants is not sufficient to ensure a significantly greater sensitivity to DDT. The very fact of the persistence of these mutants during the course of selection is, of course, evidence that they were not seriously deleterious under the conditions then present.

One could imagine that selection for susceptibility could simply mean selection for general weakness, and that selection for tolerance

to DDT could mean selection for general fitness. If this were so then one might expect fixation, or high frequency, of many mutants in the Low Lines and elimination of mutants in the High Lines. In fact the High Lines had as many or more mutants (both in kind and frequency) as did the Low Lines. The persistence of these mutants was caused by some selective effect more subtle than simple hardiness.

(iii) *Comparison with other stocks*

DDT resistant stocks obtained from other laboratories were tested to provide a comparison with the products of selection described in this study. Fig. 5 shows the results of subjecting all available selected

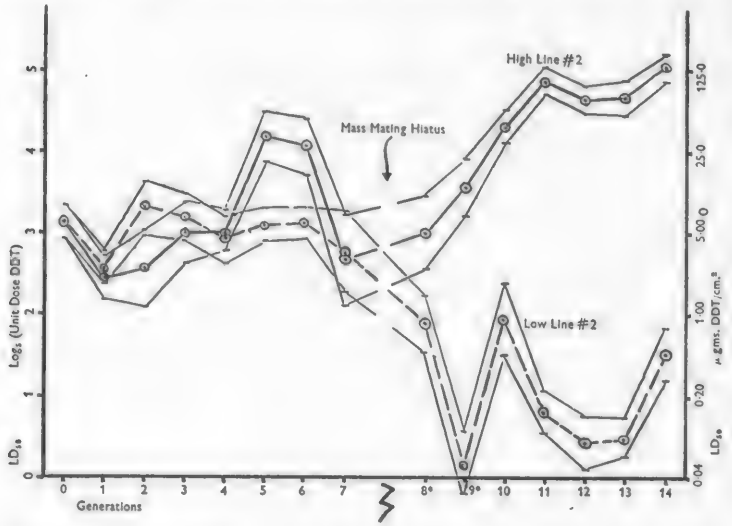
High Line #2
Mass Mating Hiatus
Low Line #2
Log$_2$ (Unit Dose DDT)
LD$_{50}$
μ gms. DDT/cm.^{2}
LD$_{50}$
Generations
0 1 2 3 4 5 6 7 8* 9* 10 11 12 13 14
0 1 2 3 4 5
125·0 25·0 5·00 1·00 0·20 0·04

FIG. 3.—The course of selection in High and Low Lines #2. Mean and 95 per cent. confidence limits of LD_{50}'s of Sibships in each generation of sib-selection.

* Defective tests, not strictly comparable to the rest.

stocks to the same culture and testing conditions. This comparison was of particular interest because there was doubt as to the relative degree of resistance achieved in different laboratories (i.e., Tsukamoto, 1958 ; Milani, 1957).

It is clear from fig. 5 that the sib-selected Low Lines are much less tolerant of DDT than the unselected Cage 1 or any others shown, though the unselected M-5 ; Cy Pm ; Sb/Ubx^{130} stock (not illustrated) was even more sensitive. One might suppose that the Low Lines represent an approach to the maximum sensitivity consistent with good viability and fertility—traits which are not characteristic of the M-5 ; Cy/Pm ; Sb/Ubx^{130} stock.

The values shown for Cages 2 and 3 (in fig. 5) are those of the final two-month test period in each case. These values were chosen because they were obtained at the same time as most of the values for the other stocks. The Hikone-R strain which was selected by DDT food medium from an already resistant wild caught strain (Ogaki and Tsukamoto,

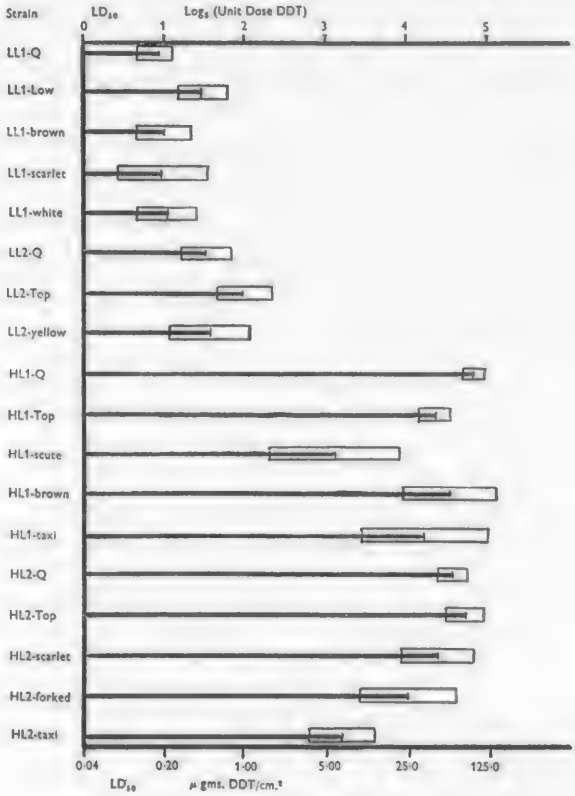

FIG. 4.—A comparison of the results of testing DDT tolerance of a number of strains and sub-strains selected for resistance and susceptibility to DDT. Mean and 95 per cent. confidence limits illustrated. (Q indicates stock made up from a representative group at the final selected generation. Top indicates stock made up from most favourable 5 progenies of the final selected generation. Low indicates stock made up from most susceptible 5 progenies of the final selected generation. Loci responsible for indicated mutant phenotypes unknown.)

1957) seems to have about the same degree of tolerance to DDT as the High sib-selected Lines. The Brown eye-R stock was selected by Crow and has been maintained in small cultures for a number of years without further selection. Its level of resistances at the time of this

study was still much above that of the control and intermediate between Cages 2 and 3.

The stocks SySM-1, SyS-1002, SyS-102 and ORS-1001 were selected by King (1954, 1957) using an aerosol containing DDT as the selective agent. All of the selection was direct, survivors of a given treatment were used as parents of the next generation (as was also the case with the Hikone-R, Brown eye-R, and Cage #2 and #3

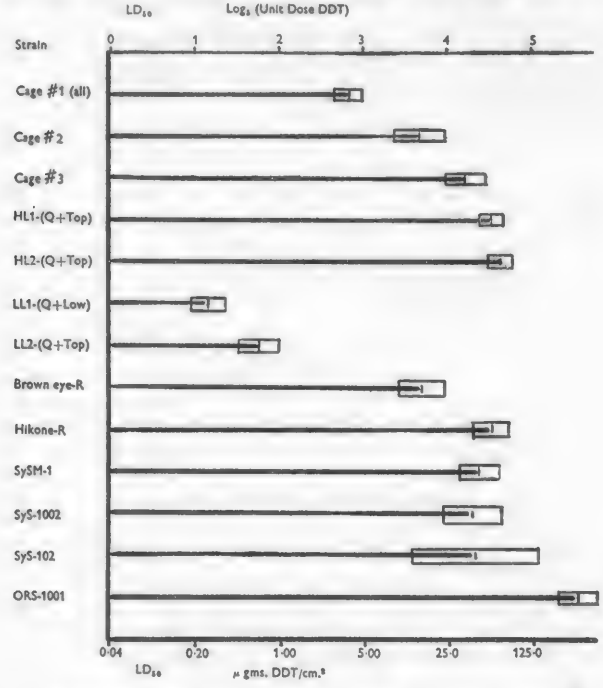

FIG. 5.—A comparison of the results of testing a number of different strains of *Drosophila melanogaster* Meigen for DDT tolerance. Mean and 95 per cent. confidence limits illustrated. ((Q+Top), (Q+Low), etc. indicate combined results for non-mutant stocks from indicated selected Line.)

stocks). The effectiveness of the sib-selection is most clearly shown here, where the sib-selection lines are seen to have become as, or more, tolerant of DDT than all of the direct selection stocks, except ORS-1001.

4. GENETIC BASIS OF RESISTANCE

The High and Low sib-selected Lines, the population cage stocks, the Brown eye-R, and Hikone-R stocks were outcrossed to M-5 ; Cy/Pm ; Sb/Ubx^{130} stock and the different phenotypic classes of hybrid offspring were tested. Fig. 6 (*a* and *b*) gives graphic comparison of the results.

Probably the most obvious point in this comparison is that the Hikone-R resistance factors are clearly dominant over the susceptibility factors in the M-5 ; Cy/Pm ; Sb/Ubx^{130} stock. In fact the hybrids seem to show a degree of heterosis, in that some of the phenotypic classes have a higher mean LD_{50} than did the parental Hikone-R stock.

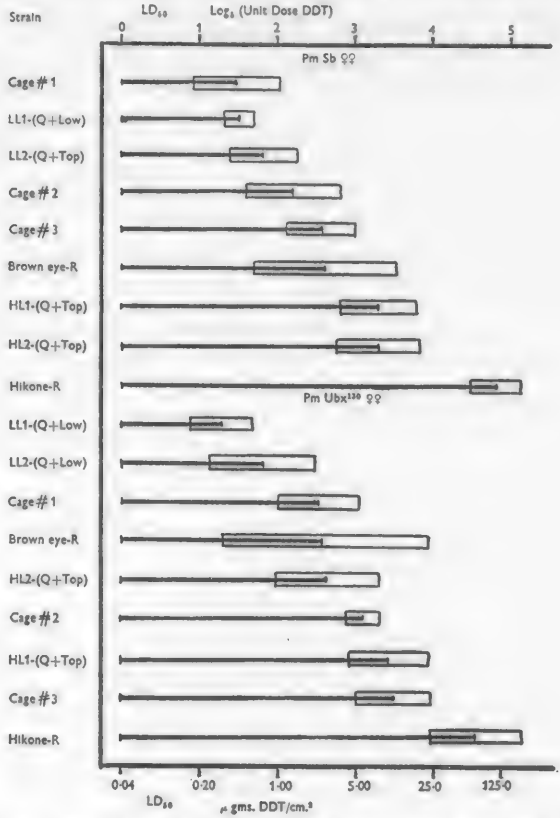

FIG. 6*a*.—The results of testing F_1 ♀♀ offspring of various strains outcrossed to the M-5 ; Cy/Pm ; Sb/Ubx^{130} stock. Grouped by similar phenotypes (Pm Sb, Pm Ubx^{130}). Mean and 95 per cent. confidence limits illustrated.

The comparisons of the other stocks are less clear. The great degree of variation within each group confuses the relative positions. The changes in relative rank, where the difference is significant, between the different phenotypic classes gives evidence of the difference in genetic basis of the resistant stocks. The interaction with the various mutant chromosomes was apparently quite different for many of the stocks. The sib-selected High stocks and the Cage 3 stock show changes in relative position in each phenotypic class. The unselected

Cage 1 stock was generally about the same as the Low sib-selected Lines in these outcrosses. Apparently the susceptibility factors were completely recessive and the resulting tolerance was simply a reflection of heterosis between genetically diverse stocks.

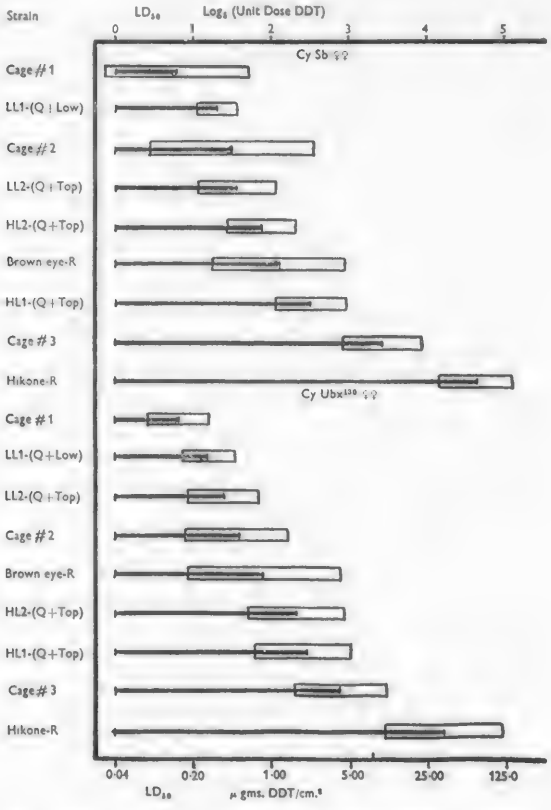

FIG. 6*b*.—The results of testing F_1 ♀♀ offspring of various strains outcrossed to the M-5 ; Cy/Pm ; Sb/Ubx^{130} stock. Grouped by similar phenotypes (Cy Sb, Cy Ubx^{130}). Mean and 95 per cent. confidence limits illustrated.

5. DISCUSSION

It is evident from figs. 1, 2, and 3 that both direct and sib-selection have been effective. The plotted curves are very similar, in general, to selection curves relating to quite different characteristics in *D. melanogaster* and other species. In this study the period of rapid change in the populations came at different times in the different lines, but was relatively early when compared with the lines developed by King (1957). Some of King's lines showed little response to selection until

they had undergone 20 or 30 generations of selection. They then rose rapidly (in resistance) to high values. King's ORS-1001 finally achieved an LD_{50} nearly 35 times that of the control line after 73 generations of selection, and his SyS-1001 and SyS-1002 lines achieved values of 16 and 20 times that of the control lines after some 60 generations. These lines were selected at the 50 per cent. level by direct exposure to DDT aerosols. Their changes compare well with the following values of the difference of the sib-selected lines from their control: HL1, 34x; HL2, 35x; LL1, 1/3x; LL2, 1/8x; in 15 generations for the first Lines and 14 generations for the second Lines.

The reason for the shorter selection times for the sib-selection lines can probably be found both in their origin and in the selection method. By sib-selection those progenies from parents capable of producing resistant genotypes were selected, but non-resistant genotypes were also included by the same token. In King's lines only resistant phenotypes survived to reproduce. Thus in the sib-selection lines genes which might, in a different genotypic environment, produce resistance, were kept in the population. In the direct selection in King's lines only strongly resistant phenotypes survived, genes with the mere potentiality to produce resistance in another genotype being quickly eliminated. King's lines originated from sources of relatively little variability, The sib-selection and population cage lines reported here were started with all of the variability obtainable in 26 different sources at hand. With greater initial genetic variability, and a breeding system that tended to maintain variability, it is not too surprising that selection proceeded more rapidly in this study. There appears to be some indication (figs. 3 and 4) that the "Mass Mating Hiatus" may have allowed more effective recombination in the sib-selected lines and thus contributed to the post-Hiatus gains made by some of the lines.

The results of the outcrosses to the M-5 ; Cy/Pm ; Sb/Ubx^{130} stock indicated that each of the resistant lines had a somewhat different genetic basis, at least as indicated by degree of dominance and interactions with the various tester chromosomes. This result is in agreement with King's (1957) conclusions, and seems to show again, if indeed it need be shown, that populations of similar origin will respond in different ways to similar selective pressures, and can achieve similar phenotypic endpoints with different genotypic bases. The outcrosses also show that the sib-selected high lines appear to show less dominance than the mass-selected Cage 3. This perhaps indicates that sib-selection was more likely to pick out recessives than was the mass selection in a population cage.

Crow (1957) has proposed that in a large randomly mating population selection would be strongly in favour of genes which conferred good general viability and a maximum average resistance in a variety of genotypic backgrounds. The sib-selection lines were selected primarily for resistance, and for viability only to the extent of being able to produce a minimum of 20 to 25 females, in each progeny.

Since the sib-selection lines were relatively small in size—only 25 effective pairs of parents in each generation (thus a maximum of 100 samples of each chromosome)—there would be greater likelihood of selecting chromosomes which had favourable specific interactions. Some support for the view that the selection in the sib-selected lines was not strongly for general fitness is obtained by comparing the values of resistance of each of the High Lines in the final selected generation to their mean value during the following two months, when they were mass cultured and tested, but not selected. High Line 1 had a final selection value for the LD_{50} of 133·5, but a mean value for the following two months of 57·9. High Line 2 had similar values of 137·0 and 67·8. These represent a loss of about one-half of the resistance, on the average, during this period. The fact that the lines still retained a resistance higher than Cage 3 and most of the other lines tested may reflect simply the short time available for reversion to have occurred. However, the loss may only reflect the difference of culture conditions between the relatively uncrowded vials, during sib-selection, and crowded bottles during the later period.

By sib-selection it has been possible to show that a single population can contain the genetic factors for either sensitivity or resistance to an environmental agent, and that those factors can be selected and concentrated without contact with the agent. This proof of pre-adaptation in this case does not of course eliminate the possibility of post-adaptation under some other circumstances. The proof does, however, make the hypothesis of post-adaptation superfluous.

It has been shown by Lüers (1953) that DDT is not mutagenic in *D. melanogaster*, thus eliminating the possibility that direct exposure might produce mutants, some of which might contribute to a post-adaptation. This is supported also by the fact that the directly selected lines were generally inferior to the sib-selected lines in resistance, which would hardly be expected if exposure to DDT were producing mutations that contributed to the resistance. Crow (unpublished) has shown that doses of DDT which do not kill, will not select for, or in any way increase, resistance. Thus for this species, as for some bacteria (Cavalli-Sforza and Lederberg, 1956), it is not necessary to propose any mechanism for the appearance of resistance in populations other than selection of pre-existing variation normally present in those populations.

6. SUMMARY

A heterogeneous stock of *Drosophila melanogaster* Meigen was proliferated in population cages and sub-populations extracted for selection. Samples of each of an initial group of 46 pair mating progenies were tested in vials lined with DDT impregnated paper to determine resistance to kill. The untested sibs from the 20 most resistant and the 20 least resistant progenies were used to make up 50 pair matings to start each of the prospective lines. Similar testing and selection was carried out on subsequent generations for each line.

Selection was carried on for 15 generations. At the end of this period the median lethal doses for the resistant lines were approximately 125 and 625 times as great as those for their respective paired susceptible lines, and more than 30 times as great as the median lethal dose for the control stock. Two population cages were subjected to direct contact with DDT and tested during the same period. The resistance developed by sib-selection compares favourably with the resistance of lines, from this and other laboratories, that were produced by direct selection. The pre-adaptation hypothesis is sufficient to account for the resistance of the sib-selected lines.

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INBREEDING AS A RESULT OF IMPRINTING

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1. INTRODUCTION

The phenomenon of imprinting is now well known in many birds, in fish, and apparently in some insects (Thorpe, 1956). In geese, which show the most extreme form of this behaviour, a man or large moving object first seen by the newly-hatched gosling will be accepted by the bird as its parent, and later on, as a member of its species to which the sexual behaviour will be attached. Recently Huxley (1955) has suggested that the imprinting of the young by their mothers may account for the assortive mating between the Blue and Lesser Snow Goose, both apparently phases of *Anser cærulescens*. Assortive mating also appears likely (O'Donald, 1959*a*) among the phases—pale, intermediate and dark—of the Arctic Skua, *Stercorarius parasiticus*. It may therefore be of some interest to examine the rate of inbreeding which such a system might produce. More generally, it would seem possible that a slight tendency to marry a partner similar in certain respects to the parent of the opposite sex may be present even in man. We shall first of all, therefore, discuss the case where imprinting is complete; and secondly the situation in which only a proportion of the population imprints, the remainder mating at random.

2. THE GENERATION MATRIX

We shall assume that both homozygotes and heterozygote are recognizable in a population in which two alleles are segregating. There are four different types of mating :—

(1) The offspring of matings of like homozygotes only reproduce similar mating types.

(2) For matings homozygote × heterozygote, however, equal numbers of homozygous and heterozygous offspring will be produced. Of the homozygous offspring, assuming mating is equally likely with either of the two parental genotypes, homozygote × homozygote and heterozygote × homozygote matings should occur with equal frequency. Similarly, the heterozygous offspring should give equal numbers of heterozygote × homozygote and heterozygote × heterozygote matings. Thus a mating AA × AB is expected to yield in the next generation $\frac{1}{4}$AA × AA, $\frac{1}{2}$AA × AB and $\frac{1}{4}$AB × AB.

(3) Heterozygote × heterozygote matings produce offspring all of

which will mate with the heterozygote: the mating type $AB \times AB$ gives $\frac{1}{4}AA \times AB$, $\frac{1}{2}AB \times AB$ and $\frac{1}{4}BB \times AB$.

(4) Finally, matings of unlike homozygotes, producing all heterozygous offspring, yield equal numbers of the two homozygote $\times$ heterozygote mating types.

Thus the following generation matrix may be built up :—

Mating type		Frequency					
		P_n	Q_n	R_n	S_n	T_n	U_n
$AA \times AA$	P_{n+1}	1	.	$\frac{1}{4}$	.	.	.
$BB \times BB$	Q_{n+1}	.	1	.	$\frac{1}{4}$	.	.
$AA \times AB$	R_{n+1}	.	.	$\frac{1}{2}$	.	$\frac{1}{4}$	$\frac{1}{2}$
$BB \times AB$	S_{n+1}	.	.	.	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{2}$
$AB \times AB$	T_{n+1}	.	.	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	.
$AA \times BB$	U_{n+1}	.	.	.	.	.	.

This matrix has the latent roots 1 (repeated), $\frac{1}{2}$, $(2+\sqrt{2})/4$, $(2-\sqrt{2})/4$ and 0. Here the dominant latent root is $(2+\sqrt{2})/4$. To find the principal component of frequency corresponding with this root, the columns of the matrix (using the notation of Fisher, 1949) are read as homogeneous linear functions of the coefficients $a \ldots . e$. Thus for the latent root $\lambda=(2+\sqrt{2})/4$, we have the equations,

$$-\tfrac{1}{4}\sqrt{2}.c+\tfrac{1}{4}.e=0$$
$$-\tfrac{1}{4}\sqrt{2}.d+\tfrac{1}{4}.e=0$$
$$\tfrac{1}{4}.c+\tfrac{1}{4}.d-\tfrac{1}{4}\sqrt{2}.e=0,$$

so that

$$c=d=e/\sqrt{2}.$$

Hence the principal component is

$$D=R+S+T.\sqrt{2}.$$

Similarly, the principal components of frequency may be built up for the remaining latent roots. We thus have :—

Principal component	Latent Root
$A=4P+3R+S+2T$	1
$B=4Q+R+3S+2T$	1
$C=R-S$	$\frac{1}{2}$
$D=R+S+\sqrt{2}.T$	$(2+\sqrt{2})/4$
$E=R+S-\sqrt{2}.T$	$(2-\sqrt{2})/4$
$F=U$	0

3. THE RELATIVE FREQUENCIES AND COMPLEXITIES

If the principal components C and E are put equal to zero (that is to assume that sufficient generations have passed to ensure that only the principal component corresponding with the dominant latent

root is of importance), we have

$$R=S$$
$$R+S=\sqrt{2}.T$$

Hence

$$\begin{aligned} R+S+T &= (1+\sqrt{2})T \\ &= \sqrt{2}(1+\sqrt{2})S \\ &= \sqrt{2}(1+\sqrt{2})R \end{aligned}$$

Thus the relative frequencies of the heterogeneous matings are :—

Mating	Symbol	Relative frequency
AA×AB	R	$1/(2+\sqrt{2})$
BB×AB	S	$1/(2+\sqrt{2})$
AB×AB	T	$1/(1+\sqrt{2})$

Inserting the relative frequencies of R, S and T in the principal component D, we find

$$D=4/(2+\sqrt{2}).$$

Hence in standard measure,

$$4D=(2+\sqrt{2})R+(2+\sqrt{2})S+(2+2\sqrt{2})T.$$

The coefficients of R, S and T form the complexities of the heterogeneous mating types.

Mating type	Symbol		Complexity in standard measure
AA×AB	R	$2+\sqrt{2}$	0·853553
BB×AB	S	$2+\sqrt{2}$	0·853553
AB×AB	T	$2+2\sqrt{2}$	1·207107
		$\div 4$	

The complexities measure the value for each mating type which multiplied by $\{(2+\sqrt{2})/4\}^n$ gives the probability of this mating type still being present n generations later.

4. THE RATE OF INBREEDING

The rate of progress towards matings involving only like homozygotes depends essentially on the dominant latent root. Fisher's method of comparing different systems of inbreeding is to use $(-\log_e \lambda)$: then the number of generations required to make a fixed amount of progress is measured by $1/(-\log_e \lambda)$. Thus for selfing, sibbing and imprinting, we have :—

	Selfing	Sibbing	Imprinting
λ	0·5	0·809017	0·853553
$-\log_e \lambda$	0·69315	0·21194	0·15835
$1/(-\log_e \lambda)$	1·443	4·718	6·315

5. INCOMPLETE IMPRINTING

The case now to be considered is that in which a proportion α of the population imprints, while the remainder mate at random. Using the same symbols as before for the different matings, we have the following set of recurrence relations.

$$\begin{aligned}
P' &= \alpha(P+\tfrac{1}{4}R)+(1-\alpha)(P+\tfrac{1}{2}R+\tfrac{1}{4}T)^2\\
Q' &= \alpha(Q+\tfrac{1}{4}S)+(1-\alpha)(Q+\tfrac{1}{2}S+\tfrac{1}{4}T)^2\\
R' &= \alpha(\tfrac{1}{2}R+\tfrac{1}{4}T+\tfrac{1}{2}U)+2(1-\alpha)(P+\tfrac{1}{2}R+\tfrac{1}{4}T)(\tfrac{1}{2}R+\tfrac{1}{2}S+\tfrac{1}{2}T+U)\\
S' &= \alpha(\tfrac{1}{2}S+\tfrac{1}{4}T+\tfrac{1}{2}U)+2(1-\alpha)(Q+\tfrac{1}{2}S+\tfrac{1}{4}T)(\tfrac{1}{2}R+\tfrac{1}{2}S+\tfrac{1}{2}T+U)\\
T' &= \alpha(\tfrac{1}{4}R+\tfrac{1}{4}S+\tfrac{1}{2}T)+(1-\alpha)(\tfrac{1}{2}R+\tfrac{1}{2}S+\tfrac{1}{2}T+U)^2\\
U' &= 2(1-\alpha)(P+\tfrac{1}{2}R+\tfrac{1}{4}T)(Q+\tfrac{1}{2}S+\tfrac{1}{4}T),
\end{aligned}$$

where $P=P_n$, $P'=P_{n+1}$, etc.
Then we have, putting $P+Q=1-(R+S+T+U)$, $\frac{1}{2}R'+\frac{1}{2}S'+T'$ $=(1-\frac{1}{2}\alpha)(\frac{1}{2}R+\frac{1}{2}S+\frac{1}{2}T+U)+\frac{1}{2}\alpha(\frac{1}{2}R+\frac{1}{2}S+T)$. But $\frac{1}{2}R+\frac{1}{2}S+T$ is the frequency of heterozygotes: letting this value be v, we obtain

$$\begin{aligned}
v' &= \tfrac{1}{2}\alpha v+(1-\tfrac{1}{2}\alpha)(\tfrac{1}{2}R+\tfrac{1}{2}S+\tfrac{1}{2}T+U)\\
&= v+(1-\tfrac{1}{2}\alpha)(U-\tfrac{1}{2}T).
\end{aligned}$$

Hence at equilibrium, when $v'=v$, we have $U=\frac{1}{2}T$. Putting $U'=\frac{1}{2}T'$ and $U=\frac{1}{2}T$, we obtain

$$\begin{aligned}
&\tfrac{1}{2}\alpha(\tfrac{1}{2}R+\tfrac{1}{2}S+T)+(1-\alpha)(\tfrac{1}{2}R+\tfrac{1}{2}S+T)^2\\
&=4(1-\alpha)(P+\tfrac{1}{2}R+\tfrac{1}{2}U)(Q+\tfrac{1}{2}S+\tfrac{1}{2}U),
\end{aligned}$$

or

$$\tfrac{1}{2}\alpha v+(1-\alpha)v^2=4(1-\alpha)uw,$$

where u and w are the frequencies of the two homozygotes. Then we have

$$\alpha v/2(1-\alpha)=4uw-v^2,$$

or

$$\alpha(1-F)/4(1-\alpha)=F,$$

where $F=1-v/2pq=(4uw-v^2)/4pq$ is the inbreeding coefficient and p and q are the gene frequencies. Hence at equilibrium the population consists of

$$(p^2+Fpq)AA+2pq(1-F)AB+(q^2+Fpq)BB,$$

where $F=\alpha/(4-3\alpha)$. Thus when p and q are equal, an equilibrium is established at

$$v_e=2(1-\alpha)/(4-3\alpha).$$

6. RATE OF APPROACH TO EQUILIBRIUM

To obtain the rate of approach to equilibrium it is necessary to find a recurrence relation in v only. This is made simpler if we put $P=Q$ and $R=S$, which is equivalent to assuming equality of gene

frequency. By this device, the recurrence relations can be re-written in terms involving only R, T and U:

$$R' = \tfrac{1}{2}\alpha(R+\tfrac{1}{2}T+U)+(1-\alpha)(R+\tfrac{1}{2}T+U)\{1-(R+\tfrac{1}{2}T+U)\}$$
$$T' = \tfrac{1}{2}\alpha(R+T)+(1-\alpha)(R+\tfrac{1}{2}T+U)^2$$
$$U' = \tfrac{1}{2}(1-\alpha)\{1-(R+\tfrac{1}{2}T+U)\}^2.$$

Then we have, in generation $(n+2)$

$$v'' = \tfrac{1}{2}\alpha v' + (1-\tfrac{1}{2}\alpha)(R'+\tfrac{1}{2}T'+U').$$

But

$$R'+\tfrac{1}{2}T'+U' = \tfrac{1}{4}\alpha v + \tfrac{1}{2}\alpha(R+\tfrac{1}{2}T+U)+\tfrac{1}{2}(1-\alpha)$$

and

$$R+\tfrac{1}{2}T+U = (v'-\tfrac{1}{2}\alpha v)/(1-\tfrac{1}{2}\alpha).$$

Thus the recurrence relation required is

$$v'' = \alpha v' + \tfrac{1}{8}\alpha(2-3\alpha)v + \tfrac{1}{2}(1-\alpha)(1-\tfrac{1}{2}\alpha).$$

It may be noted that v_e satisfies this equation.

Putting $v = \{2(1-\alpha)/(4-3\alpha)+x\}$ gives a recurrence equation in x which does not involve a constant term, namely

$$x'' = \alpha x' + \alpha x(2-3\alpha)/8.$$

Thus the rate of approach to equilibrium will be determined by the roots of the equation

$$y^2 - \alpha y - \alpha(2-3\alpha)/8 = 0.$$

The roots are

$$\tfrac{1}{2}\alpha \pm \tfrac{1}{2}\sqrt{\alpha(1-\tfrac{1}{2}\alpha)},$$

of which the dominant root is of course the one with the positive sign. It may be noted that if $\alpha = 1$, we have the case of complete imprinting and the dominant root is $\frac{1}{2}+\frac{1}{2}\sqrt{\frac{1}{2}}$ or $(2+\sqrt{2})/4$ as was obtained from the matrix.

Some numerical results of this solution are shown in table 1.

TABLE 1

α	v_e	λ	$-\log_e \lambda$	$1/(-\log_e \lambda)$
0·01	0·49874	0·0548748	2·9027	0·3445
0·1	0·48649	0·204110	1·5891	0·6293
0·2	0·47059	0·312132	1·1643	0·8589
0·3	0·45161	0·402488	0·91009	1·099
0·4	0·42857	0·482843	0·72806	1·374
0·5	0·40000	0·556186	0·58665	1·705
0·6	0·36364	0·624037	0·47155	2·121
0·7	0·31579	0·683542	0·38047	2·628
0·8	0·25000	0·746410	0·29248	3·419
0·9	0·15385	0·801781	0·22092	4·527
0·99	0·019417	0·848536	0·16424	6·089

Thus for small values of α the equilibrium point is very close to that for random mating and equilibrium is rapidly attained.

7. ASSORTIVE MATING IN THE ARCTIC SKUA

The genetics of the colour phases of the Arctic Skua are not, apparently, as simple as would permit a direct application of the calculations put forward here. The situation is complicated by the semi-dominance of the dark allele, by classification difficulties, and by the likelihood of some form of disturbance, possibly viability, in the data (O'Donald, 1959*b*).

TABLE 2

Locality and year	P×P	P×D	D×D	χ^2
Fair Isle, 1954-58. .	10	34	64	1·99177
Foula, 1948. . .	2	7	9	0·02880
Unst, 1948 . . .	4	15	36	0·79781
Yell and Fetlar, 1948 .	5	25	36	0·00784
Noss, 1946 . . .	7	9	21	5·04175
Caithness, 1953 . .	1	11	9	0·27320
Unst, 1959 . . .	9	19	43	5·13699
Total . . .	38	120	218	10·23997

The possibility of assortive mating among the phases has already been discussed. Additional data have now been collected from Unst, Shetland, lending support to this conclusion. The whole of the data now available are given in table 2, where 2×2 contingency tests of independence have been used to give χ^2 for one degree of freedom with Yates' correction. " D " represents dark and " P " pale.

TABLE 3

		Chicks		Total
		Like	Unlike	
Parents {	Like	12	6	18
	Unlike	5	5	10
Total		17	11	28

By summing the separate values of χ^2 for each locality, we obtain χ^2_{total} for seven degrees of freedom. Subtracting the χ^2 value for the total data, we find $\chi^2_{heterogeneity}$ for six degrees of freedom. Thus $\chi^2_{total} = 13{\cdot}2782$ and $\chi^2_{het.} = 3{\cdot}0382$.

The evidence thus appears fairly strongly in favour of assortive mating, and there is no evidence of heterogeneity.

At Fair Isle, however, data have also been collected involving birds of the next generation. This has been made possible by an extensive ringing programme begun in 1948 by K. Williamson, the

first warden of the bird observatory, and continued from 1957 by the present warden, Peter Davis, and myself. Owing to the great increase in the Arctic Skua colony through these years, it has been possible to recapture a number of breeding birds that were ringed as chicks. These data have been arranged in a 2×2 contingency table (table 3) according to whether the mating of the offspring is like-phased or unlike-phased in relation to a similar classification of the parental mating.

Fisher's exact test (Fisher, 1954) for the probability of this and more extreme sets of values gives a one-tailed probability of 0·3205. These data thus provide no evidence for imprinting ; but in view of the only slight tendency to assortive mating in the population as a whole, a large body of data would presumably be needed to establish whether this depended upon imprinting.

8. SUMMARY

The rate of inbreeding as a result of the imprinting of young by their parents is considered, firstly in the case of complete imprinting, and secondly where only a proportion of the population imprints. Complete imprinting leads to homozygosity with an equivalent generation time of 6·315 as against 4·718 for sib-mating and 1·443 for selfing. Thus complete imprinting takes 1·338 times as long as sib-mating and 4·377 times as long as selfing to achieve the same degree of homozygosity.

In the more realistic case where only a proportion of the population imprints, an equilibrium is established and a polymorphism will result. Where the proportion of imprinting individuals is small, however, the equilibrium point is very close to that for random mating and equilibrium is rapidly attained.

Assortive mating in the Arctic Skua is discussed, but more data are required to establish whether imprinting is a significant factor affecting it.

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GENETIC CONTROL OF TRYPTOPHAN PYRROLASE IN *DROSOPHILA MELANOGASTER* AND *D. VIRILIS*

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1. INTRODUCTION

STUDIES in micro-organisms (Giles, Partridge and Nelson, 1957; Yura, 1959; Yanofsky and Crawford, 1959) have shown that mutant genes can cause synthesis of altered enzymes, either completely inactive or active at a reduced rate (Fincham, 1957). Differences between allelic mutants may result from the production of enzymes altered to different extents (Giles, Partridge and Nelson, 1957; Yanofsky and Crawford, 1959*; Pateman and Fincham, 1958). In some cases such mutant proteins are strongly affected by environmental conditions, as temperature or ion concentration (Horowitz and Fling, 1953; Maas and Davis, 1952). Suppressor mutations are useful in discriminating between different alleles, since they can restore the enzymatic activity of some mutants only (Yanofsky and Bonner, 1955). In *D. melanogaster* many allelic series are known, the components of which show different phenotypic traits or different behaviour when coupled with a suppressor gene (for a recent review see Carlson, 1959). In this organism it seemed of interest to analyse a case of allelism and the action of a suppressor mutation in terms of the function of the gene product.

Allelism at the *vermilion* (*v*) locus (I—40·0±) was chosen for the analysis. The *v* locus controls formylkynurenine synthesis from tryptophan and consequently the formation of brown eye pigment, an end product of the tryptophan-kynurenine pathway in insects. When formylkynurenine is added to the food of *v* larvæ, the eye colour of the adult flies is normal (Green, 1952). In a previous paper (Baglioni, 1959) it was shown that the enzyme tryptophan pyrrolase‡, which catalyzes the oxidation of tryptophan to formylkynurenine, is missing in the alleles v^1 and v^{36f}. Green (1954) showed that v^1 and v^{36f} recombine and interpreted them as pseudoalleles. Moreover the v^1 allele is suppressed by a suppressor mutation, which does not cause production of brown pigment in flies homozygous for v^{36f} (Green, 1952). Other alleles, which behave like v^1, are designated as v^s (*vermilion* suppressible alleles); those behaving like v^{36f} are designated as v^u (*vermilion* unsuppressible alleles). An additional criterion can be

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‡ This enzyme was previously designated as tryptophan peroxidase-oxidase (Knox and Mehler, 1950); recently Tanaka and Knox (1959) have pointed out that the enzyme is more accurately described by the term tryptophan pyrrolase.

used in distinguishing v^s from v^u alleles ; when larvæ are submitted to partial starvation, at emergence the v^s flies show brown pigmentation of the eye, while the v^u do not (Green, 1954).

For a comparative genetic analysis also two unlinked mutants of *D. virilis*, *cardinal* (*cd*) and *vermilion*^{48b} (v^{48b}) were studied. Both mutants fail to synthesize the brown eye pigment, but become like wild type when supplied with formylkynurenine during larval life (Green, 1952); both accumulate non-protein tryptophan (Green, 1949) and are non-autonomous in transplantation experiments, like the *v* mutants of *D. melanogaster*. The two *D. virilis* mutants can, however, be differentiated by the same tests used with the *v* alleles of *D. melanogaster* : *cd* flies show a wild type phenotype in the larval starvation test, while v^{48b} do not (Green, 1954). When *cd* and v^{48b} eye discs are transplanted into *D. melanogaster* larvæ with a suppressor (no genes homologous to the *v* suppressor of *D. melanogaster* are known in *D. virilis*), the *cd* eye discs become normally pigmented while the v^{48b} discs do not (Price, 1949). Thus *cd* behaves like the v^s alleles of *D. melanogaster*, while v^{48b} behaves like the v^u alleles.

For these reasons Green (1955) suggested that mutants which occur as members of a pseudoallelic system in *D. melanogaster* can occur as unlinked mutants in the *D. virilis* species.

2. MATERIALS AND METHODS

Strains : mutant strains of *D. melanogaster* v^1, v^{36f}, *su*^{2}*-s* v^1, *su*^{S2}*-pr-v*, *su*^{3}*-s cv v f* and *su*^{2}*-s w*^{a} *cv t* were obtained from the California Institute of Technology ; the strains *su*^{2}*-s* v^1 ; *bw* and *su*^{2}*-s* v^{36f} ; *bw* were kindly supplied by Dr M. M. Green. Mutant strains of *D. virilis* v^{48b}, *cd* and *es* were obtained from the Department of Zoology, University of Chicago. The wild type strains of *D. melanogaster* and *D. virilis* and all the mutant strains of *D. melanogaster* not mentioned above, were from the species collection of our Institute.

Enzyme preparation : flies were bred on a standard corn-meal-agar medium at 25° ; flies isolated within a day after emergence were transferred into vials with fresh medium, where they were aged three to five days before being used. After being aged the flies were collected and immediately used for assay of tryptophan pyrrolase activity. Crude enzyme extracts were prepared by homogenising the flies in four volumes by weight of a solution 0·14 M in KCl and 0·0025 M in NaOH. After centrifugation (20 min. at 16,000 g.) the clear supernatants were used for enzyme assay.

Enzyme assay : incubation mixtures contained 1 ml. of 0·2 M phosphate buffer *p*H 7, 0·3 ml. of L-tryptophan 0·3 M and 1 or 2 ml. of enzyme extracts (final volume 3·3 ml.). The samples were incubated for 3 hours at 37° in a Brown shaker and the reaction was stopped by the addition of 1·1 ml. of 20 per cent. trichloroacetic acid. After filtration the kynurenine produced was determined by the Bratton-Marshall reaction, as described previously (Baglioni, 1959). Duplicate determinations were carried out for each experiment and the activities calculated by difference with blanks in which tryptophan was omitted.

Mutants of *D. melanogaster* were tested in combination with *brown* (*bw*) ; homogenates from these strains give lower blank values, because the red eye pigment, the synthesis of which is prevented by the gene *bw*, absorbs at 560 mμ (the wavelength at which the diazotised kynurenine was read). The mutant *cd* of *D. virilis* was tested in combination with *eosinoid* (*es*), which prevents in this species the formation of the

red pigment. Since Glassman (1956) has shown that kynurenine formamidase, which catalyses the hydrolysis of formylkynurenine to kynurenine, is present in normal amount in all the strains tested, it was assumed that formylkynurenine formed from tryptophan was quantitatively transformed into kynurenine.

Special medium : feeding experiments were performed by adding tryptophan to the medium, 5 per cent. brewers' yeast and 1·5 per cent. agar. Vials were autoclaved before use and L-tryptophan added, before the medium had solidified, to a final concentration of 0·25 per cent. 20-30 eggs rinsed in ethanol and wiped with filter paper were transferred in each vial.

3. TRYPTOPHAN PYRROLASE ACTIVITY OF *D. melanogaster* STRAINS

Tryptophan pyrrolase activity was determined in two wild type strains of *D. melanogaster* and in mutant strains of this species, in which the synthesis of the brown pigment was blocked at different steps of the tryptophan-ommochrome pathway. The results are reported in table 1. The results of a previous investigation (Baglioni, 1959),

TABLE 1

Tryptophan pyrrolase activity of wild type and mutant strains of Drosophila melanogaster

Strain	Mean activity ± standard error	No. of experiments
wild type (*Oregon R*) .	133±11	6
wild type (*Pavia*) .	82±12	3
v^1 ; *bw* . . .	14±2	6
v^{36f} ; *bw* . .	10±2	6
bw . . .	138±15	3
st ; *bw* . . .	235±25	6
cn bw . . .	192±22	6
bw ; *cd* . . .	110±11	3
v^1 ; *bw*+v^{36f} ; *bw** .	9±2	3

The tryptophan pyrrolase activity is expressed as μ moles $\times 10^{-3}$ of kynurenine formed by one gram of flies in one hour at 37°.

* In these experiments equal amounts of the extracts of the two strains were mixed before the incubation.

concerned with the tryptophan pyrrolase activity of the pseudoalleles v^1 and v^{36f} are also reported for comparison.

The tryptophan pyrrolase activity of the *Oregon R* wild type flies is greater than that of the *Pavia* wild type strain; this is probably due to genetic differences between the two lines. The mutants *cinnabar* (*cn*) and *scarlet* (*st*) displayed a considerably greater enzyme activity than the wild type. The concentration of the enzyme may perhaps increase in mutants which block tryptophan metabolism ; the evidence is not conclusive, since the strains examined have different genetic backgrounds. In this connection it is important to point out that the tryptophan pyrrolase activity of *cardinal* (*cd*) flies, in which brown pigment formation is incompletely blocked but strongly lowered, is quite normal.

When extracts of v^1 ; *bw* and v^{36f} ; *bw* were incubated together, no increase in activity was observed. It seems therefore unlikely that the *v* pseudoalleles act on two successive steps of a chain of reactions.

4. THE ACTION OF THE SUPPRESSORS OF *VERMILION*

The suppressors of *vermilion* (I—0·0+) are assumed to be members of an allelic series ; these suppressor genes have no visible effect when alone and can be recognised only when combined with the mutant genes they suppress. Three suppressor strains were tested for tryptophan pyrrolase activity in combination with the suppressible mutants v^1 : su^2-*s*, su^3-*s* and su^{S2}-*v*-*pr*. The suppressor su^2-*s* was also tested in combination with v^1 ; *bw*, with v^{36f} ; *bw* and not combined with a *v* mutant in the strain su^2-*s* w^a *cv t*.

The results of tryptophan pyrrolase determination in suppressor strains are reported in table 2.

A partial recovery of enzyme activity takes place in the presence of

TABLE 2

Tryptophan pyrrolase activity of suppressor of v *strains*

Strain	Mean activity ± standard error	No. of experiments
su^2-*s* v^1 . . .	26 ± 6	6
su^2-*s* v^1 ; *bw* . .	26 ± 3	9
su^2-*s* v^{36f} ; *bw* . .	8 ± 1	3
su^3-*s* *cv* v^1 *f* . .	22 ± 3	6
su^{S2}-*v*-*pr* v^1 . .	19 ± 4	6
su^2-*s* w^a *cv t* . .	103 ± 9	5
su^2-*s* v^1 + v^1 ; *bw** .	24 ± 3	3
su^2-*s* v^1 + v^{36f} ; *bw** .	18 ± 2	3

The tryptophan pyrrolase activity is expressed as μ moles $\times 10^{-3}$ of kynurenine formed by one gram of flies in one hour at 37°.

* In these experiments equal amounts of the extracts of the two strains were mixed before the incubation.

suppressor genes ; in the unsuppressed strain su^2-*s* v^{36f} ; *bw* a tryptophan pyrrolase activity greater than that of the v^{36f} ; *bw* strain was not observed.

When enzyme extracts of strain su^2-*s* were mixed and incubated together with those of the v^1 ; *bw* and v^{36f} ; *bw* strains, no increase of tryptophan pyrrolase activity beyond the activities determined separately was observed. Thus the action of the suppressor genes seems to be limited to the restoration of the tryptophan pyrrolase of the v^1 mutant *in vivo*.

The action of the suppressor genes seems to be qualitatively identical ; they increase the rate of tryptophan oxidation in the v^1 strain but not in the v^{36f}. The quantitative significance of the results was examined by a *t* test (see table 3). The action of the suppressor genes on the tryptophan pyrrolase activity of the v^1 mutant is small, although significant in at least two of the three strains examined.

The tryptophan pyrrolase activity of the strain su^2-*s* w^a *cv t* seems to be like that of the wild type ; the suppressor genes apparently have no effect on the enzyme activity of the v^+ allele.

The introduction of the mutation *cinnabar* (*cn*) in the strain su^2-*s* v^1 ;

bw gave further evidence that the suppressors of *v* are concerned with the activation of tryptophan pyrrolase in the v^1 mutant and do not provide an alternate pathway of tryptophan metabolism. The mutant *cn* is known to lack the ability to oxidise kynurenine to 3-OH-kynurenine. Crossing *cn bw* flies and su^2-*s* v^1 flies to a lethal balanced strain and crossing the progeny in the proper way, the strain su^2-*s* v^1 ; *cn bw* was selected. The su^2-*s* v^1 ; *cn bw* flies are white eyed. Since

TABLE 3

Statistical test of the significance of the differences between the mean tryptophan pyrrolase activity of the strain v^1 ; bw *and the mean activities of the suppressed* v^1 *strains*

Strain	*t* values	Probability range
su^2-*s* v^1 ; *bw* .	3·245	0·01-0·001
su^3-*s cv* $v^1 f$.	2·280	0·05-0·02
su^{s2}-*v*-*pr* v^1 .	1·119	0·2-0·3

the phenotypic manifestation of the suppressor gene is hidden by the mutation *cn*, it can be concluded that the suppressor of *v* does not provide a way to bypass the metabolic block of the *cn* mutants and that its action is limited to the immediately preceding metabolic step, namely the oxidation of tryptophan to kynurenine.

Attempts have also been made to determine the action of the

TABLE 4

Cross	Genotype of the female progeny	Phenotype
su^2-*s* v^1 ; *bw* × v^1 ; *bw* . .	$\frac{su^2\text{-}s}{+}$ v^1 ; *bw*	Diluted brownish
su^2-*s* v^1 ; *bw* × su^2-*s* v^{36f} ; *bw* .	su^2-*s* $\frac{v^1}{v^{36f}}$; *bw*	Brown (lighter than su^2-*s* v^1 ; *bw*)

suppressor gene su^2-*s* on heterozygous v^1/v^{36f} females and of the heterozygous suppressor gene on v^1 ; *bw* flies.

Proper crosses were done and the phenotype of the female progeny examined for eye pigmentation ; the results are reported in table 4. The heterozygous su^2-*s* $\frac{v^1}{v^{36f}}$; *bw* females are suppressed to an intermediate level. Their pigmentation is intermediate between that of su^2-*s* v^1 ; *bw* flies and that of v^1 ; *bw*. This fact supports the idea that the two pseudoallelic genes v^1 and v^{36f} work quite independently from each other and that the suppression is due only to the amount of the product of the v^1 gene present.

The heterozygous suppressor has a relatively weak effect on the pigmentation of v^1 ; *bw* flies ; it is not, however, a true recessive since heterozygous $\frac{stl^2\text{-}s}{+}$ v^1 ; *bw* females can be easily distinguished from v^1 ; *bw* flies.

5. DIFFERENTIATION OF *VERMILION* PSEUDOALLELES

Valadares and Jacquot (1952) observed a slight pigmentation in *v* flies when the larvæ were grown in a sterile medium supplemented with L-tryptophan. We were able to show that the mutant v^1 produces some brown pigment when fed tryptophan, while v^{36f} does not. These mutants were tested in combination with *brown* (*bw*). The larvæ were supplied with a special medium, in which tryptophan was present at a concentration of 0·25 per cent. (see *Materials and Methods*).

The pigmentation of the tryptophan-fed v^1 ; *bw* flies was clearly appreciable, although the quantity of the pigment formed was much less than in wild type flies. The identity of the pigment produced by the tryptophan-fed v^1 ; *bw* flies with the brown pigment of the wild type was investigated with a chromatographic test. The flies were prepared for chromatography according to the methods of Hadorn and Mitchell (1951) and the chromatograms were then developed in the solvent collidine/0·5 M $KH_2{}^PO_4$ (2/3). A spot identified as xanthommatin, according to Butenandt and Neubert (1955), was observed in chromatograms obtained from wild type and in smaller amount in the chromatograms from tryptophan-fed v^1 ; *bw* flies.

6. TRYPTOPHAN PYRROLASE ACTIVITY OF *D. VIRILIS* STRAINS

The results of tryptophan pyrrolase determinations in *D. virilis* strains are shown in table 5. The tryptophan pyrrolase activity of the

TABLE 5

Tryptophan pyrrolase activity of D. virilis *strains*

Strain	Mean activity ± standard error	No. of experiments
wild type . .	86±6	8
v^{48b} . .	6±0·5	6
cd . . .	49±3	8
cd ; *es* . .	84±4	8
wild type+v^{48b}*	47±3	3
wild type+*cd** .	63±3	3
cd+v^{48b}* . .	26±2	3

The tryptophan pyrrolase activity is expressed as μ moles $\times 10^{-3}$ of kynurenine formed by one gram of flies in one hour at 37°.

* In these experiments equal amounts of the extracts of the two strains were mixed before the incubation.

v^{48b} flies is reduced to a very low level ; the activity of the *cd* mutant is only slightly lower than that of wild type, or as in the case of the *cd* ; *es* flies, equal to that of the wild type.

When enzyme extracts of the strains examined were mixed before incubation, no increase nor inhibition of the tryptophan pyrrolase activity was recorded. In all cases the activity corresponded, within the limits of random fluctuations, to the mean of the activities determined separately.

7. DISCUSSION

The incubation with L-tryptophan of extracts of mutant strains v^1 ; *bw* and v^{36f} ; *bw* yields less than 1/10 of the amount of diazotisable amine produced by the wild type. The specificity of the Bratton-Marshall reaction being relatively low, it cannot be established whether the small amount of diazotisable material formed by the *v* pseudoalleles is kynurenine or not.

No spot corresponding to kynurenine appears on paper chromatograms of the concentrated incubation mixtures (Baglioni, 1959). But this does not rule out that the diazotisable material may be kynurenine, since this test is not sensitive enough. Alternatively the diazotisable material may be some other aromatic amine produced from tryptophan by the extracts.

It is indeed impossible to establish if v^1 and v^{36f} flies are completely devoid of tryptophan pyrrolase activity, although it seems likely that these mutant strains lack this enzyme.

The present data exclude the possibility that the two *v* alleles examined are concerned with successive steps in a sequence of reactions, as suggested for some *D. melanogaster* alleles by the Lewis (1956) hypothesis of the physiology of pseudoallelism. Our results are instead consistent with the interpretation of pseudoallelism proposed by Pontecorvo (1956) on the basis of studies on white alleles of *D. melanogaster* and on biotin mutants of *Aspergillus nidulans*. According to Pontecorvo (1956) pseudoalleles are mutated at sites, separable by crossing-over, of a large physiological gene. They are therefore truly alleles.

Gene *v* seems to consist of only one cistron, since complementation between alleles has never been observed. The mutations v^1 and v^{36f} implicate changes at sites, which are a few recombination units apart from one another, but a single physiological function, namely the production of the enzyme tryptophan pyrrolase, is affected. The difference in the behaviour of *v* alleles shown by Green's (1952 ; 1954) results and by the present results, are to be ascribed to the alteration of the gene product induced by the mutation. The mutant v^{36f} forms a greatly altered gene product, inactive in conditions which allow a partial recovery of the mutant v^1. It is quite probable that the action of the suppressors of v^1 lead to the activation of an enzyme less severely altered than that of the v^{36f} alleles. The suppressor genes increase slightly the tryptophan pyrrolase activity of v^1, but the effect of the suppressor genes might be more marked during the pupal life, when the brown pigment is more actively synthetised. The low activity of the

extracts makes it hard to investigate the tryptophan pyrrolase activity at various developmental stages.

The hypothesis that the suppressors of *v* act by activating an inactive enzyme is supported by the following considerations :—

(i) The non-specificity of suppressor genes, which like the suppressor of *v* suppress several unrelated mutant genes, could be better explained by supposing that the suppressor modifies an environmental factor, which prevents the function of the mutated genes, than by claiming complicated metabolic relationships. The suppressors of *v* are known to suppress beside the v^s alleles the mutant *sable*, *speck* and *purple*. According to Pontecorvo (1958) " the suppressor acts by modifying some cell conditions to which the changed products of several genes are all sensitive, though only those changed in particular ways."

(ii) The partial suppression of v^s mutants by treatments such as larval starvation or tryptophan administration proves that the enzyme of these mutants can function when environmental conditions are changed in particular ways.

(iii) The heterozygous $su^2\text{-}s\frac{v^1}{v^{36f}}$; *bw* flies synthesize a much higher quantity of brown pigment than the heterozygous $v^1\ \frac{su^2\text{-}s}{+}$; *bw* flies. Thus the modification induced by the homozygous suppressor seems to be the most important condition for the suppression, while the dose of the v^1 gene present determines a quantitative response. The loci *v* of *D. melanogaster* and *D. virilis*, which are located in homologous segments of the *X*-chromosome, have an identical function since they are both concerned with the synthesis of tryptophan pyrrolase. The mutant v^{48b} is lacking this enzyme ; it cannot be established if it is completely devoid of tryptophan oxidising ability. Considerations similar to those reported above for the *v* pseudoalleles are valid also for the mutant v^{48b} of *D. virilis*.

The gene *cd* of *D. virilis* inhibits brown pigment formation by a quite different mechanism ; the brown pigment is indeed present in the ommatidia of *cd* flies in reduced amount. No explanation can be advanced so far as to the action of the *cd* gene.

8. SUMMARY

1. The tryptophan pyrrolase activity of two wild type strains of *D. melanogaster* and of several mutant strains was determined. Only the allelic mutants v^1 and v^{36f} were found to have an activity considerably lower than that of the wild type.

2. The suppressors of *v* $su^2\text{-}s$, $su^3\text{-}s$ and $su^{S\,2}\text{-}s$ were found to increase the tryptophan pyrrolase activity of the v^1 mutant but not of the v^{36f} mutant. Further evidence was provided that the action of the suppressors of *v* is concerned with the metabolic step leading from tryptophan to kynurenine and does not provide an alternate metabolic pathway.

3. The two alleles v^1 and v^{36f} can be differentiated if supplied during larval life with a medium containing high concentrations of L-tryptophan. In these conditions the mutant v^1 synthesizes brown pigment, while the v^{36f} does not.

4. The mutant v^{48b} of *D. virilis* was found to lack the enzyme tryptophan pyrrolase; the mutant *cd*, which is likewise devoid of brown pigment and responds to formylkynurenine administration, showed a normal tryptophan pyrrolase activity.

5. The results are discussed in view of the theories on pseudoallelism of Lewis (1956) and Pontecorvo (1956). There is a one locus-one enzyme relationship in the case of the loci *v* of *D. melanogaster* and *D. virilis*. The action of the suppressors of *v* is discussed and the conclusion is reached that these genes activate alternate gene products by changing some cell condition.

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NEW LOCI IN THE SEX CHROMOSOME OF THE FOWL*

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1. INTRODUCTION

THE first tentative map for the fowl's sex chromosome showed the approximate locations of seven genes (Hutt, 1936). In a pictorial map published 13 years later (Hutt, 1949) only one gene could be added (Munro's sex-linked dilution), but even for that the exact locus was uncertain, as it still is. However, the studies of Punnett (1940) permitted some helpful revision, particularly in locating the gene *Id*. Moreover, by 1949 three additional sex-linked mutations were known—naked, albinism and dwarf—but their positions in the chromosomes were not.

From tests for linkage carried out during the past 22 years, together with the contributions of Mueller (1952) and Cole (1958), it is now possible to add five new loci to the map of the sex chromosome, and to extend it considerably beyond its earlier limits.

2. THE MUTATIONS INVOLVED

The mutants here considered have all been described in some detail earlier, hence only brief notes about them should be necessary in this report. The dominant genes *B*, *S* and *K*, which cause barring, silver and slow feathering, respectively, are old friends familiar to all fowl geneticists, and were already spotted in earlier maps. The others, all of which are recessive, are as follows :—

Albinism, *al* (Mueller and Hutt, 1941). This is an imperfect albinism. Chicks have bright pink eyes when hatched, but in adults the iris is blue-grey, and barred birds show faint ghost-barring in the plumage. In the eye, melanin is restricted to the pigment layer of the retina and the retinal portions of the ciliary body and iris. Vision and viability are apparently normal.

Dwarf, *dw* (Hutt, 1949, 1959). This gene reduces the size of adult females by about 30 per cent. and that of homozygous males by about 43 per cent., but chicks that become dwarfs are of normal size at hatching. Their viability and reproduction are normal except that dwarf hens lay somewhat fewer and smaller eggs than their normal sisters.

Paroxysm, *px* (Cole, 1958). At various times after 10 days of age,

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females carrying *px* will react to sudden disturbance by throwing the head over the back, becoming rigid and beating the wings violently. Such a paroxysm lasts 10 to 15 seconds, after which the chick relaxes. None survive more than a few weeks.

Naked, *n* (Hutt and Sturkie, 1938). Affected females are bare at hatching, or show a little down, but are usually all bare by four weeks of age when the down has been lost. Some adults are bare, others almost normal except for missing or defective remiges. Homozygous males are affected less than the hemizygous females. About half the naked chicks die during the last two or three days of incubation, and others after hatching, so that without extra care only slightly more than 25 per cent. of the zygotes are raised to maturity.

Shaker, *sh* (Scott *et al.*, 1950). Affected birds show at two to five weeks of age very rapid quivering of the head and neck. Incoordination of movements becomes progressively worse and by 14 weeks most shakers cannot stand. Only a few survive to sexual maturity. Purkinje cells of the cerebellum are absent or degenerated.

3. PROCEDURES

Apart from the usual handicap that crossing-over between sex-linked genes can be measured only in gametes of the homogametic sex, linkage studies with these five genes are complicated by the facts that three of them are lethal (*px*, *n* and *sh*), one delayed in expression (*dw*), and two (*al* and *n*) prevent the manifestation of some other sex-linked genes. As doubly recessive females in some combinations could not be raised to breeding age, and in others were difficult to maintain, the general practice was to test dihybrid or trihybrid males, which were mated to normal females, usually White Leghorns or New Hampshires. From such matings only the female progeny could be used to measure crossing-over in the gametes of their sires.

Because of all these limitations, most tests were repeated several times and with different males, not only to increase the numbers of gametes tested, but also to determine whether or not consistent results could be obtained.

4. LINKAGE OF *AL*

(i) *With K.* The *K-k* alleles causing slow feathering and rapid feathering, respectively, are evident in most chicks at hatching, but are perhaps more accurately distinguished at 10 days of age. Even then, there are usually a few that are difficult to classify. In all linkage studies involving these genes, descriptions were recorded both at hatching and 10 days later. Since in most tests the chance of being a cross-over ranged only from 2 to 18 per cent., no chick was considered as such unless its description at 10 days agreed with that at hatching.

In tests not complicated by other genes, *al* showed only 1·8 per cent. crossing-over with *K* in 274 gametes from 3 males (table 1).

This same relationship was also measured in gametes from 5 males

of the genotype *Al k n*/*al K N*. These males are indicated in table 1 by asterisks (*) with one parental class. As *K* and *k* cannot be classified in naked chicks, only about half the female progeny (those carrying *N* and alive at 10 days) could be used for this test. It will be noted that, among these, one parental class, *al K N*, is over four times as frequent as the other. This disparity results from the fact that the parental class *Al k n*, being naked, would be completely unidentifiable (as *K* or *k*) if linkage of these 3 genes were complete. Gametes carrying the parental combination *Al k* can be identified only as a result of crossing-over between *k* and *n*, which yielded 53 chicks of the recognisable genotype *Al k N*. Cross-overs in 288 gametes from these 5 males numbered only 4, or 1·4 per cent.

Considering all 8 matings together, crossing-over between *al* and *K* in 562 gametes was 1·6 per cent.

(ii) *With n.* Regardless of its shortcomings for tests of linkage with other genes, *n* is very satisfactory for tests with *al*. Lethal action of *n* on developing embryos is not exerted until the last 3 days of incubation, and mostly in the last two. There is usually a shortage of naked chicks among those hatched, but, on examination of the dead-in-shell, it is easy to identify naked, albinotic and normal chicks that have died late in incubation. The first two of these classes must be females, if from normal mothers, as were all those considered in table 1. By dissection of the normal chicks, any *Al N* females among the dead-in-shell are identified. When sexes of the normal chicks that hatched are determined, one has thus a complete record for all females that were alive at 18 days of incubation.

To save space, records are not given separately here for the dead-in-shell and hatched chicks. As the data show (table 1), the observed ratios of *Al* : *al* (376 : 372) and *N* : *n* (373 : 375) are so close to the expected 1 : 1 that they might well be viewed with suspicion.

Crossing-over between *al* and *n* in 748 gametes from 6 males was 17·6 per cent., and the separate determinations for each male were reasonably consistent.

(iii) *With dw.* As dwarfs cannot all be accurately classified until four or five months of age, there is always the difficulty that mortality before that age reduces numbers somewhat, and the risk that some other mutation, when combined with dwarfism, might cause disproportionate reduction of the double recessive class. Fortunately that did not happen in these tests, and there was even an excess of albinotic dwarfs above the number for the other cross-over class (table 1). Crossing-over in 270 gametes from the two males tested was 7 per cent.

(iv) *Order of the genes al, K and n.* It was evident that *n* is more remote from *al* than *K* is, but the question whether or not the latter gene lies between the other two had to be answered. The 5 triply heterozygous males starred in table 1 were useful for that purpose.

If their three linked genes were arranged *k* 1·6 *Al* 17·6 *n*/*K* 1·6 *al*

17·6 *N*, then recognisable (*N*) single cross-overs between *K* and *al* would be *k al N* and the number expected in 288 gametes would be 4·6. Double cross-overs, *K Al N*, should number theoretically only 0·8.

TABLE 1

Tests for linkage of al

Year	Male	Coupling or repulsion	Female progeny				Crossing-over, per cent.
	(1) *al* and *K*		*Al k*	*al K*	*Al K*	*al k*	
1945	M 01	R	89	81	2	1	1·7
1950	T 51	R	19	10	0	0	0·0
1954	B 36	R	40	30	2	0	2·8
		Totals :	148	121	4	1	
			269		5		1·8
1941	H 40	R	5*	18	1	0	4·2
1951	U 14	R	14*	74	1	0	1·1
1951	U 16	R	10*	34	1	0	2·2
1951	U 17	R	19*	67	0	0	0·0
1954	B 25	R	5*	38	1	0	2·3
		Totals :	53*	231	4	0	
			284		4		1·4
	For all 8 males : Parental : 553 Cross-overs : 9						1·6
	(2) *al* and *n*		*Al n*	*al N*	*Al N*	*al n*	
1941	H 40	R	34	30	9	9	22·0
1951	U 14	R	67	81	15	18	18·2
1951	U 16	R	43	38	12	11	22·1
1951	U 17	R	82	74	18	13	16·6
1954	B 25	R	41	43	6	7	13·4
		Totals :	267	266	60	58	18·1
1954	B 28	C	7	7	42	41	14·4
	For all 6 males : Parental : 616 Cross-overs : 132						17·6
	(3) *al* and *dw*		*Al dw*	*al dW*	*Al Dw*	*al dw*	
1945	M 01	R	80	64	4	9	8·3
1952	V 55, 56	R	55	52	2	4	5·3
		Totals :	135	116	6	13	
	For both males : Parental : 251 Cross-overs : 19						7·0

* For explanation of apparent deficiency of this parental class, see context.

With the alternative order, *Al-k-n*/*al-K-N*, these classes are reversed, and single cross-overs between *al* and *K* should yield 4 or 5 *Al K N* chicks but hardly any of the *al k N* double cross-overs. The observed numbers in these two classes, 4 and 0, make the second arrangement the more likely one.

5. LINKAGE OF *DW*

In addition to the tests of *dw* with *al* considered in the previous section, the former gene was also tested with *B* (barring), *S* (silver) and *K* (table 2).

TABLE 2

Tests for linkage of dw

Year	Male	Coupling or repulsion	Female progeny				Crossing-over, per cent.
	(1) *dw* and *B*		*B Dw*	*b dw*	*B dw*	*b Dw*	
1942	J 03	C	14	17	8	16	43·6
1944	M 54	C	48	33	60	52	58·0
1944	M 51	C	19	29	27	36	56·8
		Totals :	81	79	95	104	
			160		199		55·4
	(2) *dw* and *S*		*Dw S*	*dw s*	*Dw s*	*dw S*	
1942	J 03	C	34	23	4	1	8·1
1942	H 33	C	31	26	3	2	8·1
1943	K 37	C	88	78	6	8	7·8
		Totals :	153	127	13	11	7·9
1944	M 54	R	8	4	92	91	6·2
1944	M 51	R	2	3	55	53	4·4
1951	U 10	R	2	5	37	30	9·5
		Totals :	12	12	184	174	6·3
	For all 6 males : Parentals : 638 Cross-overs : 48						7·0
	(3) *dw* and *K*		*Dw K*	*dw k*	*Dw k*	*dw K*	
1942	J 03	C	33	25	2	3	7·9
1942	H 33	C	31	26	2	4	9·5
1944	M 58	C	64	90	6	3	5·5
1945	M 01	C	58	82	10	7	10·8
		Totals :	186	223	20	17	8·3
1944	M 54	R	8	2	92	93	5·1
1944	M 51	R	4	3	53	53	6·2
1944	M 57	R	3	3	70	66	4·2
1951	U 10	R	2	2	37	33	5·4
		Totals :	17	10	252	245	5·2
	For all 8 males : Parentals : 906 Cross-overs : 64						6·6

(i) *With B.* In 359 gametes from 3 males, cross-overs between *B* and *dw* numbered 55·4 per cent. As the ratios of 176 *B* : 183 *b* and 185 *Dw* : 174 *dw* are close to those of 1 : 1 expected, it seems probable that the slight excess of cross-overs above the theoretical maximum of 50 per cent. cannot be attributed to differential viability of any one class, or to errors of classification, but only to chance. These tests

showed that *dw* is not only remote from *B*, but also more likely to be beyond *K* than between *B* and *K*, and this arrangement was later verified.

(ii) *With S.* Among 686 females classified in these tests, cross-overs between *S* and *dw* numbered only 48, or 7·0 per cent., and separate determinations for the 6 males were reasonably consistent. The ratio of 350 *S* : 336 *s* fits expectation well. There are usually a few birds difficult to classify as silver or gold, but the ratios recorded in the cross-over classes indicate that any error from that source must have been insignificant.

(iii) *With K.* This relationship was measured in 970 gametes. Determinations for the 8 different males ranged from 4·2 to 10·8 per cent., the overall figure being 6·6 per cent.

6. LINKAGE OF *N*

(i) *With B.* As barring can be recognised at hatching (in chicks with extended black) by the white head spot, it was easy to get adequate numbers to test possible linkage of *B* with *n*. It is usually possible to classify coloured, naked chicks as barred or non-barred because the head spot shows as a cluster of unpigmented feather follicles. The other follicles, even without down, do show pigment. However, it was shown by early tests, and confirmed in later ones, that these genes are too far apart to show even a loose linkage. There is little point in giving separate determinations for each of the 6 males used in these tests and, accordingly, the data for all six have been combined (table 3).

They show 51·8 per cent. crossing-over in 351 gametes. The deficiency of naked chicks (202 *N* : 149 *n*) resulted in part from greater mortality in that class late in incubation, but also from the fact that *B* and *b* could sometimes not be recognised until the chicks had grown more feathers than they had at hatching. By that time their numbers were reduced. This does not affect the validity of the test for linkage because any reduction should affect equally one parental class and one cross-over class. Separate determinations for the normal chicks only and for naked chicks only are 51·9 and 51·7 per cent.

(ii) *With S.* This test was more difficult because the silver and gold alleles were seldom distinguishable at hatching in the naked chicks. Many of them died before acquiring enough feathers to show silver or gold, and, even with some comparatively well-feathered ones, classification was difficult. The more accurate measure of crossing-over between *S* and *n* is therefore obtained by considering only the parental and cross-over classes containing chicks not naked.

In 192 of these from 6 males (table 3) cross-overs numbered 41, or 21·4 per cent. Among the 62 naked chicks, 25·8 per cent. were classified as cross-overs.

(iii) *With K.* It was impossible to distinguish in naked chicks between those carrying *K* and their sisters with rapid feathering, hence crossing-over could be measured only in the females with

normal plumage. Only a quarter of the gametes from each sire could thus be utilised, but over the years it was possible to get records for 748 classifiable gametes from 16 different males. Among these 17·6 per cent. were classified as cross-overs (table 3).

Four of these males used in 1940 which had comparatively small

TABLE 3

Tests for linkage of n

Year	Male	Coupling or repulsion	Female progeny (classifiable)				Crossing-over, per cent.
	(1) *n* and *B*		*B N*	*b n*	*B n*	*b N*	
1940	6 males, as listed below	C	97	72	77	105	51·8
	(2) *n* and *S*		*S N*	*s n*	*S n*	*s N*	
1940	G 14	C	31	16	7	9	22·5*
1940	G 15	C	10	0	0	4	28·6*
1940	G 17	C	76	24	8	20	20·8*
1940	G 18	C	16	0	0	5	23·8*
1940	G 20	C	12	1	0	2	14·3*
1940	G 22	C	6	5	1	1	14·3*
		Totals :	151	46	16	41	21·4*
	(3) *n* and *K*		*K N*	*k n*	*K n*	*k N*	
1938	D 76	C	39			12	23·5
1938	D 83	C	40			5	11·1
1939	G 17	C	71			19	21·1
1940	G 14	C	32			8	20·0
1940	G 15, 18 20, 22	C	53	Not classifiable as *K* or *k*		11	17·2
1941	H 39	C	19			5	20·8
1951	U 14	C	76			13	14·6
1951	U 16	C	35			7	16·7
1951	U 17	C	67			19	22·1
1954	B 25	C	39			5	11·4
1954	B 30	C	59			10	14·5
1954	B 32	C	51			9	15·0
		Totals :	581			123	17·5
1954	B 28	R	9			35	20·4
	For all 16 males : Parental : 616 Cross-overs : 132						17·6

* In gametes carrying *N*.

families have been grouped together in the table. Among the other 12 males, recorded cross-overs varied from 11·1 to 23·5. While much of this variation is attributable to chance in comparatively small numbers, and some, perhaps, to actual differences among sires in rate of crossing-over, it must be recognised that some of it may have resulted from faulty classification. There are usually a few chicks that are difficult (for this author) to classify as *K* or *k*. On the other hand, as the 17·6 per cent. figure for the *K*-*n* relationship is identical with that

for *al* and *n*, and *K* and *al* are separated only by 1·6 cross-over units, these three determinations are in close agreement, and, as will be shown later, must indicate fairly accurately the loci for *al*, *K* and *n*.

7. LINKAGE OF *SH*

By kindness of Dr. C. D. Mueller, then at Kansas State College, we received in 1952 six males so bred that for each the chance of being heterozygous for the gene *sh* was better than 50 per cent. Two of these were mated in 1953 with albinos, and four were tested (by artificial insemination) with naked females. Four of the six proved to be *K sh*/*k Sh*, and these provided that year some evidence on distance between those two loci. Among their sons, further testing revealed one that carried *sh* and *al*, and two carrying *sh* and *n*. These three were then used in 1954 to provide data on the linkage relations of *sh* with *al*, *K* and *n* (table 4). As before, crossing-over could be measured only in their daughters.

TABLE 4

Tests for linkage of sh

Year	Male	Coupling or repulsion	Female progeny				Crossing-over, per cent.
	(1) *sh* and *al*		*Al Sh*	*al sh*	*Al sh*	*al Sh*	
1954	B 26	R	20	13	45	36	28·9
	(2) *sh* and *K*		*K Sh*	*k sh*	*K sh*	*k Sh*	
1953	A 17, 18	R	2	3	11	5	23·8
1953	A 20	R	2	4	6	10	27·3
1953	A 21	R	3	0	4	11	16·7
		Totals :	7	7	21	26	
			14		47		23·0
1954	B 30	R	7	9	51	1*	23·5
1954	B 32	R	8	8	44	0	26·7
		Totals :	15	17	95	1	
			32		96		25·0
	For all 6 males : Parentals : 143 Cross-overs : 46						24·3
	(3) *sh* and *n*		*N Sh*	*n sh*	*N sh*	*n Sh*	
1954	B 30	R	8	2	60	37	9·3
1954	B 32	R	8	9	52	27	17·7
		Totals :	16	11	112	64	
			27		176		13·3

* For explanation of this chick, see context.

(i) *With al.* In 114 gametes from the one male tested, cross-overs numbered 33, or 28·9 per cent. There was a slight deficiency of

albinos, particularly in the double-recessive (cross-over) class, but not more than could have occurred by chance.

(ii) *With K.* The four males found in 1953 to carry *sh* also proved to be heterozygous for *K* and *k*. As these matings were made on only a limited scale, comparatively few female offspring were available, but among 61 of these there were 23 per cent. cross-overs. These matings were uncomplicated by the presence of other sex-linked genes.

The two males used in 1954 were of the genotype *K N sh*/*k n Sh*, and they produced daughters as follows :—

K N sh	Parental	95
k N sh	Cross-over, *K-n*	17
K N Sh	Cross-over, *N-sh*	15
k N Sh	Double cross-over	1
k n Sh	Parental	64
K n Sh	Cross-over, *K-n*	
k n sh	Cross-over, *n-sh*	11
K n sh	Double cross-over	

Separate data for each male are given in table 4. The number of *N* offspring given there is one less than that for the same matings in table 3 because one chick died before an age at which it could show the shaker mutation. Considering only the first 4 classes listed above (since *K* and *k* cannot be distinguished in the naked chicks of the other four groups), and counting the lone double cross-over between *K* and *sh* as a parental gamete with respect to those two genes, cross-overs between K and *sh* numbered 32 in 128 gametes, or 25·0 per cent.

This figure agrees well with that of 26·4 per cent. determined by Mueller (1952), who measured crossing-over between *K* and *sh* in 87 gametes.

(iii) *With n.* Lethal action of *n*, either late in incubation, or after hatching and before an age at which *sh* could be manifested, considerably reduced the numbers of naked chicks from these matings, as the ratio of 128 *N* : 75 *n* shows. There is no indication, however, that the naked shakers were more adversely affected than those not naked. Accordingly, assuming that, apart from chance fluctuation in comparatively small numbers, the parental and cross-over classes carrying *n* were reduced in equal proportions, all four classes can be utilised to measure crossing-over. On this basis there were 13·3 per cent. cross-overs in 203 gametes. Separate determinations for *N* and *n* chicks only yielded 12·5 and 14·6, respectively.

(iv) *Double cross-overs.* Only one among the 128 *N* chicks listed above was classified as *k N Sh*, i.e. the result of double crossing-over between *K* and *sh*. With distances *K* 17·6 *n* 13·3 *sh* the expected number of such chicks (without any interference) was three.

8. LINKAGE OF K AND S

Before proceeding to construction of a map for the sex chromosome, some reconsideration is necessary of the linkage relations of *K* with other genes. The earlier results of several workers, when added to his own, led Punnett (1940) to place *K* remote from *B*, below *S*, and showing about 15 per cent. crossing-over with the latter gene. In this author's earlier map (Hutt, 1949) its locus was put at 11 cross-over units below *S*. However, as Punnett pointed out, there was enough variation in the determinations by different investigators to justify some doubt about the validity of an estimate derived from the pooled data. In one case, although 576 chicks were classified, the number in

TABLE 5

Tests for linkage of K *with* B *and* S

Year	Male	Coupling or repulsion	Chicks				Crossing-over, per cent.
	(1) *B* and *K*		*B K*	*b k*	*B k*	*b K*	
1944	M 51	R	52	66	57	55	51·3
1944	M 54	R	118	112	99	75	56·9
		Totals :	170	178	156	130	
			348		286		54·9
	(2) *S* and *K*		*S K*	*s k*	*S k*	*s K*	
1942	H 33	C	47	40	0	0	0·0
1942	J 03	C	56	43	2	0	2·0
1944	M 51	C	139	136	5	3	2·8
1944	M 54	C	203	215	6	8	3·2
1951	U 10	C	32	39	0	3	4·1
		Totals :	477	473	13	14	
			950		27		2·8

one cross-over class was nearly five times that in the other, where approximate equality was expected.

More recently, Jaap (1956) found in 1596 chicks that cross-overs between *K* and *S* numbered only 20, or 1·3 per cent. In the studies here reported, crossing-over between these two genes was 2·8 per cent. in 977 gametes, and the separate determinations for the 5 males were fairly consistent (table 5).

This figure would have been somewhat higher if classification as *K* or *k* at 10 days only had been accepted as correct. The restriction that cross-overs were accepted as such only when classification at 10 days agreed with that at hatching, did lower slightly the number of cross-overs recorded. On the other hand, putting the locus of *K* at 2·8 from *S*, as our data have it, does permit an orderly plan (fig. 1) based on 11 different relationships in that end of the chromosome. The arrangement and distances shown in that plan would not be equally feasible

if the distance between *S* and *K* were 15 units or more, as the earlier determinations had it. As expected, *K* was found to be quite independent of *B* (table 5).

Some years ago Dr. E. F. Godfrey, then at the University of Tennessee, tested the relationships of *B*, *K*, and *S*, and he has kindly provided the following extensive data (not published elsewhere) for inclusion in this report.

Genes	Parental combinations	Cross-overs	
		Number	Per cent.
B and *K*	909	863	48·7
B and *S*	564	441	43·9
S and *K*	934	73	7·2

Godfrey's 7·2 per cent. and Jaap's 1·3 fit our map better than any of the earlier determinations for *K* and *S*. It is worth noting also that, whereas the cumulative data for the *B*-*S* and *B*-*K* linkages, as summarised by Punnett, would put *K* slightly closer than *S* to *B*, Godfrey's figures indicate a locus for *K* a little below *S* (4·8), a position that is in accord with all the relationships shown in fig. 1.

9. THE MAP

The various relationships determined from the data in tables 1 to 5 permit construction of the maps shown in fig. 1. There are no inconsistencies, and the two minor discrepancies are no greater than could be expected by chance in comparatively limited numbers and with genes very close together. Thus, *S* and *al* both showed the same crossing-over with *dw*, but the map separates *S* and *al* by only 1·2 units. Similarly, *al* and *K* both showed 17·6 per cent. crossing-over with *n*, but that is not surprising as the map has *al* and *K* only 1·6 units apart.

The highest cross-over percentage observed, 28·9 between *al* and *sh*, is not out of line with the total distance between these genes as determined from the sum of its component segments, which is 34·6. Presumably double crossing-over in that distance would reduce somewhat the number of detectable cross-overs between the two genes at the extreme ends. Similarly, when Mueller's figures are added to the present ones for the *K*-*sh* linkage, cross-overs in the combined data number 25·6 per cent. The map distance made up from shorter segments is 33.

In fig. 1, the map on the right shows the positions of 13 genes located thus far in the fowl's sex chromosome. The major part, that from *ko* to *S*, is unchanged from the map of 1949, except that *Sd* is shown so close to *B* that the two might be alleles. Unfortunately no studies of *Sd* and its relation to *B* have been reported since the brief abstract by Munro (1946). The distance from *S* to *sh*, as worked out from the studies here reported, covers 35·8 units and thus makes the total map distance known thus far 111·8 units. This is believed to be the longest map to date for any chromosome of a vertebrate animal.

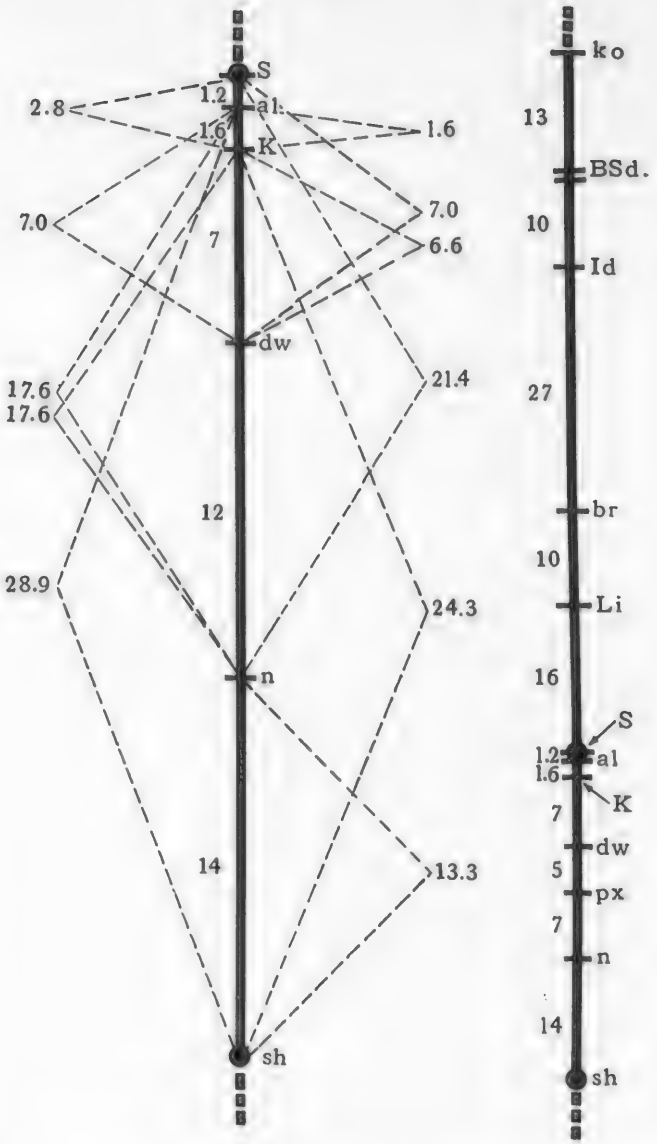

FIG. 1.—Diagrams showing (left) linkage relationships and loci of genes considered in this study, and (right) a revised, extended map for the sex chromosome.

Cole (1958) has reported that the gene *px* causing "paroxysm," a syndrome lethal in the first few weeks after hatching, shows 6 per cent. crossing-over with *n* and 10·5 per cent. with *al*. With such relations, it fits nicely in the map, as shown, at a point almost equidistant between *dw* and *n*.

There are two genes known to be sex-linked for which loci are still undetermined. These are the lethals *xl* (Goodwin *et al.*, 1950) and *j* (jittery). Godfrey *et al.* (1953) reported that *j* is apparently independent of both *B* and *K*, but there were indications in their data that the order of the genes is most likely *B-K-j*. In two separate tests, one with these three genes in the coupling phase, and the other with males of the genotype *B K j/b k J*, the smallest numbers in the progeny were found in the class that would have been double cross-overs if *K* were between *B* and *j*. The map in fig. 1, which shows altogether 33 cross-over units below *K*, makes it fairly certain that Godfrey *et al.* were right in their interpretation, and that *j* lies far enough below *sh* to show no significant linkage with *K* in the 805 gametes that they classified. It seems probable, therefore, that linkage tests with *n* and *sh* would be most likely to reveal the locus of *j*, and that the accomplishment would extend the map of the sex chromosome to more than 140 cross-over units.

In this connection it is worth recalling that the fowl's sex chromosome, which is V-shaped, ranks fifth in length and is less than half as long as the biggest chromosome (Sokolow and Trofimow, 1933). In the fowl's four largest autosomes, map distances are likely to be so great that many genes actually within one linkage group could appear to be independent. This helps to explain why so many tests for autosomal linkage have been fruitless (Warren, 1949). Only 5 autosomal groups are known, and there is no proof that any two of these do not belong together in some great, long chromosome. If the present maps for the autosomes are to be extended or filled in, it is important that any genes showing the slightest indication of linkage in small numbers of gametes be tested further on a larger scale.

10. SUMMARY

Data are given from tests for linkage which were carried out at various times since 1938 with the sex-linked genes for silver (*S*), albinism (*al*), slow feathering (*K*), dwarf (*dw*), naked (*n*) and shaker (*sh*).

From all the results, together with Mueller's figure for the *K-sh* linkage and Cole's study of paroxysm (*px*), the lower end of the sex chromosome was found to carry all these genes, with distances between them as follows : *S* 1·2 *al* 1·6 *K* 7 *dw* 5 *px* 7 *n* 14 *sh*.

For the whole chromosome, the total distance mapped thus far is 111·8, and the locations of 13 genes in that distance are shown.

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REVIEWS

DRUG RESISTANCE IN MICRO-ORGANISMS. CIBA Foundation Symposium. Ed. by G. E. W. Wolstenholme and Cecilia M. O'Connor. London: J. & A. Churchill. 1957. Pp. 352. 50s.

This symposium arose out of the desire of Sir Charles Harington, its chairman, to promote fundamental knowledge of chemotherapy, which is at present largely empirical. The aspect considered is that of drug resistance, which makes the empirical successes much less favourable from a strictly utilitarian point of view. Thus in many hospitals over half of the staphylococci causing infections are resistant to penicillin, though perhaps not to other antibiotics. The nature of such resistance and its mode of origin is the core of the discussion. Naturally, the matter is of outstanding interest to geneticists, especially in respect of the controversy between the mutation-selection and " training " schools of thought. This is argued in detail and at length by protagonists who do not seem to convince one another.

The non-genetic viewpoint is stated by Dean and Hinshelwood who " have never doubted that the essential characters of a cell are inherent in the structure of certain fundamental units . . . not . . . easily susceptible to change ". They " have never denied that structural mutations leading to increased drug resistance . . . can and do occur " but " have contested the assumption that random mutation and selection is the sole mechanism (or perhaps even the major mechanism) for adaptation to new media or for the development of drug resistance ". Consideration of this essay suggests that some of the divergence depends upon a lack of appreciation of genetical principles. They " consider that the term ' heredity ' is a rather ambiguous one to use of organisms which multiply by binary fission. . . . there is no question of parent and offspring ". In attacking the " uncompromising mutation-selection theory," it is said that " so elaborate a polygenic system is frequently assumed that the results of recombination experiments become indistinguishable from those which would be given by quantitative cytoplasmic changes ". Significant reports were given by Lederberg, Pollock and Eagle of mutation-selection situations in Hinshelwood's own material.

Every geneticist would agree that the behaviour of a cell in a given environment is dependent upon the interaction of its genotype and the environment, coloured by the previous physiological history of the cell, as shown so elegantly in the case of β-galactosidase in *E. coli*. They would also agree that there are permanent, or very persistent, hereditary changes not due to changes in structural elements like chromosomes. What is conspicuously lacking in so many of the examples, cited as non-genetic changes, is a genetic analysis of the difference between adapted and unadapted cells.

The list of participants is notable for the presence of two Russians from the Institute of Genetics, Moscow. Kossikov described " directed hereditary changes " of fermentative properties in yeast, but the changes have all the hall-marks of mutation-selection. A most interesting observation is that homozygous adapted cells have twice as much invertase as heterozygous cells. This paper is most significant for the way in which it avoids genetic interpretation.

D. G. Catcheside.

THE MATHEMATICAL THEORY OF EPIDEMICS. By N. T. J. Bailey. London: Charles Griffin. 1957. Pp. 1-194. 36s.

This is the first book to give a systematic treatment of the use of mathematical and statistical methods in understanding the spread of epidemics. The theories discussed are of interest to biometricians, and many of the results are relevant to the work of epidemiologists and medical officers of health who are concerned with the practical implications more than with the theory. Many of the models used are necessarily oversimplified, but nevertheless have given valuable results. Further development is likely to lead to useful applications only in so far as it is related to epidemiological realities. No account is taken of genetic variation in host or parasite, though the discussion of immunity may be regarded as relevant. The interest to geneticists lies chiefly in the mathematical methods employed.

Of general interest is the fact that stochastic models have given better agreement with the observed courses of epidemics than have deterministic models which ignore the probability aspects of the problem. However, several aspects of the stochastic systems cannot be managed satisfactorily and in these cases Monte Carlo methods in conjunction with an electronic computer can be of service. While the book is of marginal interest to geneticists, it should be studied by those dealing with population problems.

D. G. Catcheside.

HUMAN BIOCHEMICAL GENETICS. By H. Harris. Cambridge: University Press. 1959. Pp. 310. 37s. 6d.

This is an expansion and revision of Dr Harris's " Introduction to Human Biochemical Genetics," published in 1953, but so much has happened in a short time that whole new fields are described. The book is indeed very meaty. The first two chapters deal with basic principles, especially of population genetics applied to man. This is a good short statement, but the need for these chapters is a reflection upon the deficiencies of general biological education.

Several types of biochemical abnormality are distinguished, though these undoubtedly reflect different levels of observation or analysis rather than differences in principle. First are failures of a step in normal metabolism, these being the ultimate expression of a genetic defect in an enzyme. Many of these are different in kind from the nutritional mutations in fungi and bacteria, in which complex compounds are built from simple compounds and intermediates below a block are accumulated. In the mammal, where complex compounds are the raw materials, intermediates above blocks in catabolic processes occur.

Second are defects in the regulation of transport across membranes, e.g. the failure of renal reabsorption from the glomerular filtrate in cystinuria. Like the first failure it probably involves a defective enzyme, for active transport.

A third type is an error in the construction of a macromolecule, and it is in this field that human biochemical genetics has contributed most to genetical theory. Apart from absence or great reduction in amount of a macromolecule, as, for examples, gammaglobulin, albumin and fibrinogen, there are the highly instructive alterations of hæmoglobin. The chapter on the hæmoglobins is a very excellent account, serving to show also how shaky

is the evidence about the genetic relations of the abnormal hæmoglobins. A sound knowledge of the genetics of the alpha and beta polypeptides of hæmoglobin would make a substantial contribution to genetical theory.

The chapter on the blood group substances is also very valuable. In general, the whole book is a learned and well balanced account of the subject and should be read by biologists broadly. The final chapter on the problem of gene action is of quite general value.

D. G. CATCHESIDE.

THE THYROID GLAND. British Medical Bulletin. By Various Authors. London 1960: Pp. 89-174. 20s.

Sixteen papers including a note on " Genetic Factors " which deals with PTC Tasting relations of thyroid diseases. It takes us, one may hope, a step towards translating " the ætiological problems " of medical 19th-century speech the into genetic questions of the present day.

C. D. D.

GENETICS AND CANCER. University of Texas Press. Austin, Texas. 1960. Pp. 459. $8.50.

The above is a collection of papers delivered at the thirteenth symposium on Fundamental Cancer Research organised by the University of Texas. The material is divided into four sections with the Bertner Foundation lecture by Dr Richard Shope classified separately. The first section of three papers is entitled " The genetic theory of cancer etiology ". As is well known cancer may attack any organ in the body as may diseases such as tuberculosis and syphilis. Cancer differs from these infections in many ways including its epidemiology; for example cancer of the body of the uterus is commonest amongst well-to-do women who have borne no children whilst the opposite is true of cancer of the uterine cervix. Facts such as these have driven some pathologists to the view that it is wrong to speak of " cancer " as a unit disease and therefore we have no more reason to anticipate a common cause for neoplastic diseases than we have in the case of fevers. This somewhat nihilistic view is not popular and most people feel that malignancy depends upon certain fundamental cellular disturbances which may be precipitated by diverse environmental factors.

C. D. Darlington who opens this symposium has put forward the boldest of the unitary hypotheses. His ideas are unorthodox in two ways. He believes that the fundamental change occurs in the cytoplasm and that the altered plasmagene may become infective. One of the reasons for implicating the cytoplasm comes from a study of the chromosomes of tumours. Gross chromosomal alterations are extremely common but there is no single type of change that may be said to characterise malignancy and indeed no abnormalities may be detectible. The potential infectivity of mutated plasmagenes is anathema to some pathologists but they will be unwise to ignore Darlington's views on this account since he has much to say that should interest them.

A more orthodox view would be that agents such as carcinogenic hydrocarbons and viruses affect the same cytoplasmic components. Dr Jack Schultz takes fifteen pages to tell us that since carcinogenesis involves a change in cellular heredity and since the different systems in such heredity involve the nucleic acids of one type or another we can hardly escape some

form of the somatic mutation hypothesis. In the meantime he has discoursed very learnedly upon somatic variation in maize and Drosophila. This work is of intrinsic interest but has no visible connection with carcinogenesis at the present time. Some very eminent pathologists are ardent advocates of some form of mutation hypothesis whilst others have a somewhat reserved attitude. The fact is that none of these hypothesis add to our knowledge of such phenomena as autonomy, invasiveness or metastasis. We do know that autonomy can occur without invasiveness and that both may occur together without metastasis. Autonomy is not an " all or none " affair and full autonomy may involve a number of cellular changes. It is a matter of opinion whether it is important to call the modifications that differentiate the fully fledged cancerous cell " mutations " or whether it is better to use some less emotive term. Wollman and Jacob end the section with an excellent article on Lysogeny, Transduction and Cancer Genesis. They do not advance any general theory of carcinogenesis but they show very convincingly that studies on bacteriophage help to explain many of the most puzzling properties of oncogenic viruses.

The second section containing six papers is entitled " Fundamental Aspects of Genetics in Carcinogenesis ". The first paper is about neither of these, the second is about carcinogenesis but not genetics and the last about genetics but not about carcinogenesis. Latarjet contributes an interesting and modest article on radiation and carcinogenesis. The article by Hass and Doudney on the mechanism of bacterial mutagenesis by ultraviolet light is really excellent and their views on the possible implications for carcinogenesis most stimulating. Levan contributes an encyclopaedic article on chromosomal changes in tumours and cultured cell lines.

The third section of five papers is entitled " Gene Interaction in Neoplastic Growth ". The first article by Dr David Bonner is mainly concerned with the genetics of tryptophan synthetase in Neurospora crassa. I found it most interesting but it tells us nothing about neoplastic growth. The remaining four papers are of a high order and pertinent. Perhaps the best of all is that by Heston on the site of gene action and carcinogenesis, a subject upon which he is the acknowledged master. The Klein's work on the cytogenetics of experimental tumours is well known. The mutationists should consider the remarks they make about tumour progression.

The Bertner lecture on the Fibroma-Myxoma virus complex is a masterpiece.

The next section is entitled " Genetic Basis of Cell Resistance ". The first is by DeOme et al. and is an excellent essay on tumour progression. Dr G. W. Wooley has an article on the development of drug resistance and David Anderson on the genetics of bovine ocular carcinoma. Readers must judge for themselves why these three papers are considered under this particular heading.

The last section is on the genetics of human cancer and three of the four articles are actually concerned with this subject. Dr Andervont follows with a masterly summary of the entire proceedings.

Since we know so little about malignancy anybody may be forgiven for being hesitant before he says that any finding is unimportant. However, a symposium on Genetics and Cancer should aim at giving a survey of the present relationships existing between the two. This the organisers have failed to do and there is far too much with the most nebulous connection

with current cancer research. It may be unfair to pick out any single contribution, but Morton's article on " Methods of Study in Human Genetics " is perhaps the most glaring example of an unsuitable choice. Students of human genetics might be assumed to know the literature of their own subject and it is difficult to imagine how the analysis of autosomal and partial sex linkage in man is likely to influence Cancer Research in the forseeable future. The organisers have not helped matters by the way in which they have arranged their material. It is a pity that a book that contains so much that is good should fail when viewed as a whole. The result is a *dysposium*. The need for an up-to-date *symposium* on the relationship between Genetics and Cancer still remains.

P. A. GORER.

BOOKS RECEIVED

SCIENCE & RESOURCES. By Various Authors. Ed. by H. Jarret. Johns Hopkins Univ. Press. Oxford Univ. Press. 1960. Pp. xiii+250. 40s.

INHERITANCE OF GLIOMA. By H. J. Van Der Wiel. London: D. Van Norstrand Co. Ltd., 1960. Pp. 271. 63s.

IN AND OUT OF THE IVORY TOWER. By R. B. Goldschmidt. Univ. of Washington Press. 1960. Pp. xiii+352. $5.75.

NATURE AND MAN'S FATE. By G. Hardin. New York: Rinehart & Co., Inc. 1959. Pp. xi+375. $6.00.

THE AGE OF THE WORLD. By F. C. Haber. Johns Hopkins Univ. Press. Oxford Univ. Press. 1960. Pp. xi-303. 40s.

TERATOLOGY SOCIETY

For several years scientists interested in basic problems of congenital malformations have held informal conferences in which questions of common interest were discussed. Anatomists, biochemists, embryologists, geneticists, obstetricians, pathologists, pediatricians, plastic surgeons and others attended these conferences which were in part supported by the Association for the Aid of Crippled Children, New York, N.Y., and the Human Embryology and Development Study Section of the National Institutes of Health. With the increased interest in recent years in this area, it was felt that there was a need for a Society to hold regular meetings in which investigations concerned with etiology and morphogenesis of congenital malformations could be presented and discussed. Following the fourth teratology conference, which was held at the Memorial Sloan-Kettering Cancer Center in New York City and attended by 76 scientists from Canada, England, France, Germany and the U.S.A., "The Teratology Society" was formed for the purposes outlined above. The following officers were elected: President: Josef Warkany, M.D., Cincinnati, O.; President-Elect: James G. Wilson, PH.D., Gainesville, Fla.; Secretary-Treasurer: Marjorie M. Nelson, PH.D., San Francisco, Calif.; Recorder: Sidney Q. Cohlan, M.D., New York, N.Y.; Council: F. Clarke Fraser, PH.D., M.D., Montreal, Canada, David L. Gunberg, PH.D., Portland, Ore., and M. Lois Murphy, M.D., New York, N.Y. The National Foundation assisted in the formation of the Society with advice and financial aid. Inquiries about The Teratology Society should be directed to Dr Marjorie M. Nelson, Department of Anatomy, School of Medicine, University of California, San Francisco 22, Calif.